3D-Bioprinted PCL/GelMA-HA Scaffolds for Guided Bone Regeneration (GBR)

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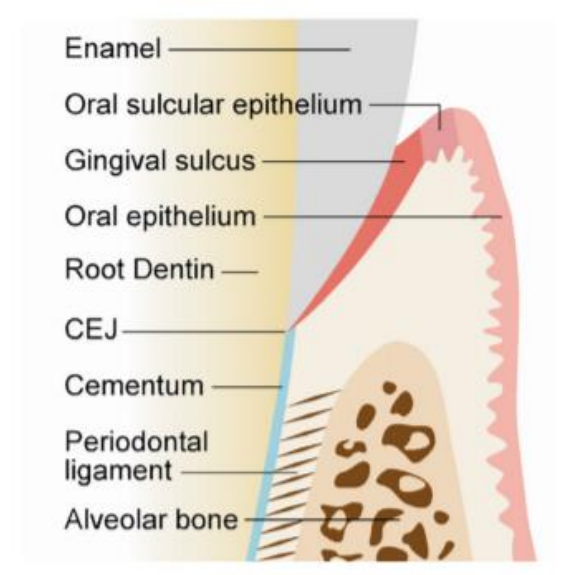
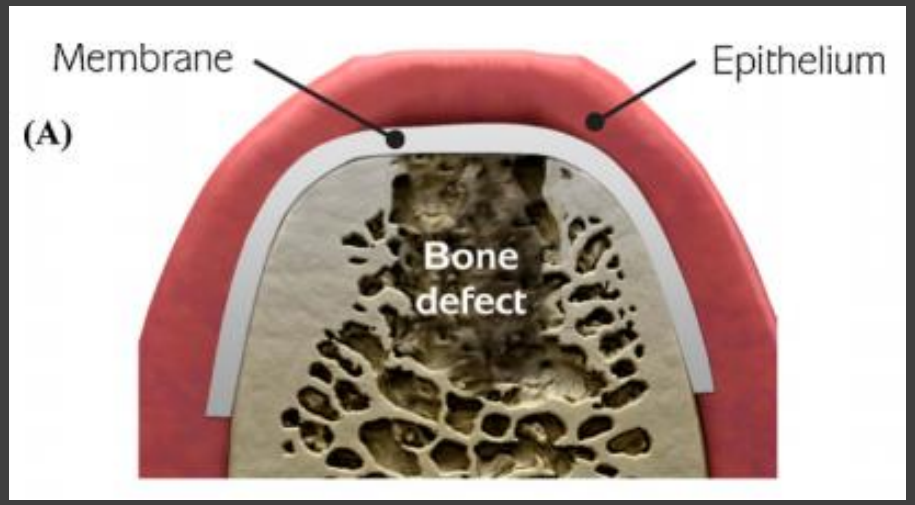




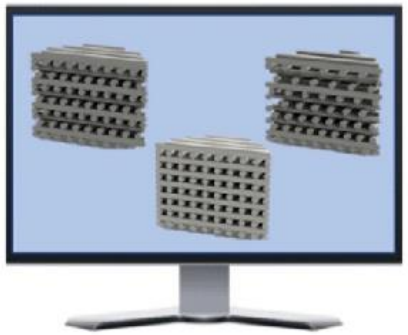
Periodontitis

- Inflammatory gum disease, erodes gums and enamel
- Objective: Synthesize a membrane to fill space and regenerate tissue
  - Must be biocompatible, biodegradable and bioactive material

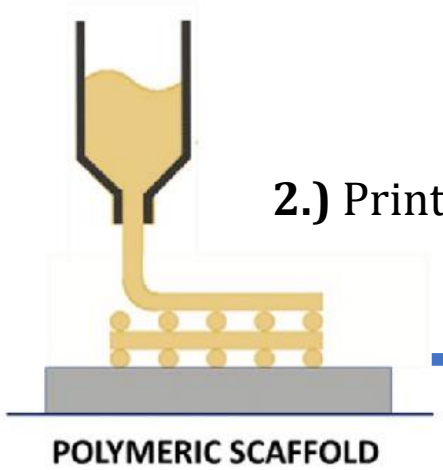
- Marco C. Bottino, Vinoy Thomas, Gudrun Schmidt, Yogesh K. Vohra, Tien-Min Gabriel Chu, Michael J. Kowolik, Gregg M. Janowski, 'Recent advances in the development of GTR/GBR membranes for periodontal regeneration—A materials perspective', Dental Materials, vol 28, Issue 7, 2012, pp 703-721. https://doi.org/10.1016/j.dental.201\-2.04.022.
- https://www.shutterstock.com/search/gum+disease+cartoon

Graphical Abstract

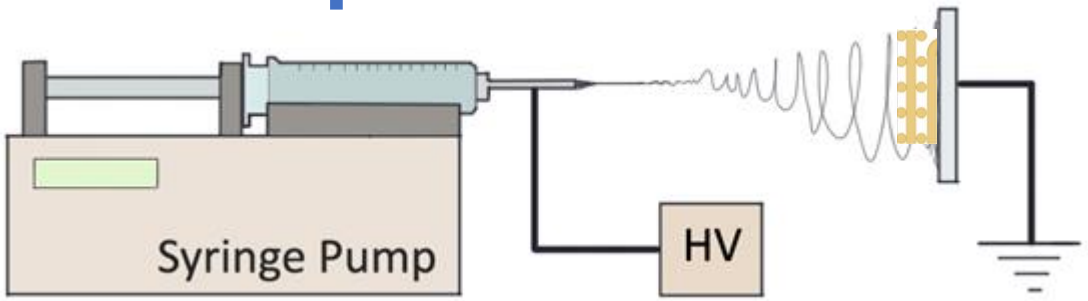
1.) Design



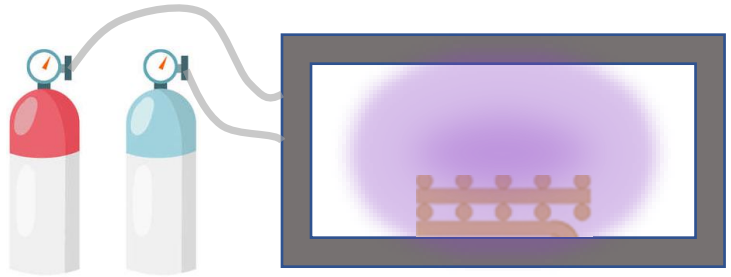
2.) Print



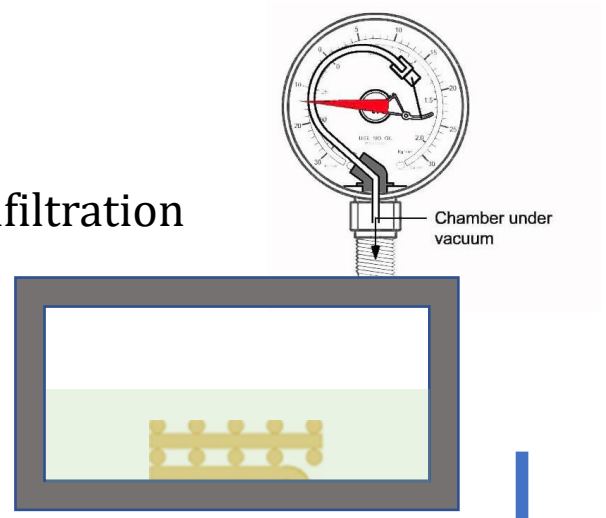
3.) Electrospinning



4.) Plasma

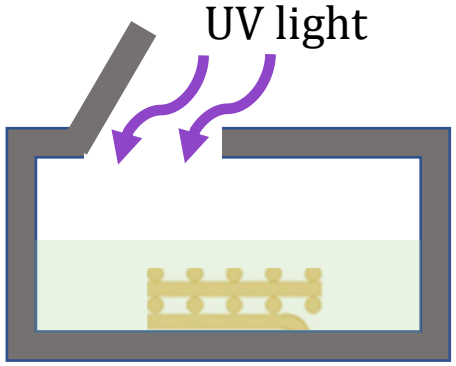


5.) Infiltration



Hydrogel (liquid)

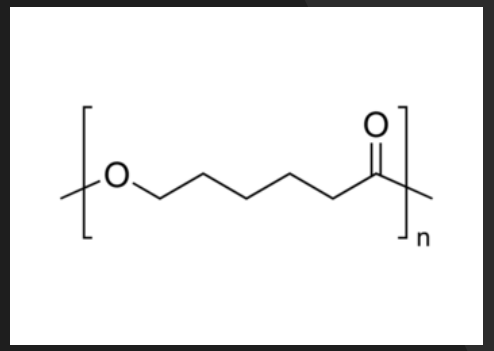
6.) Crosslinking



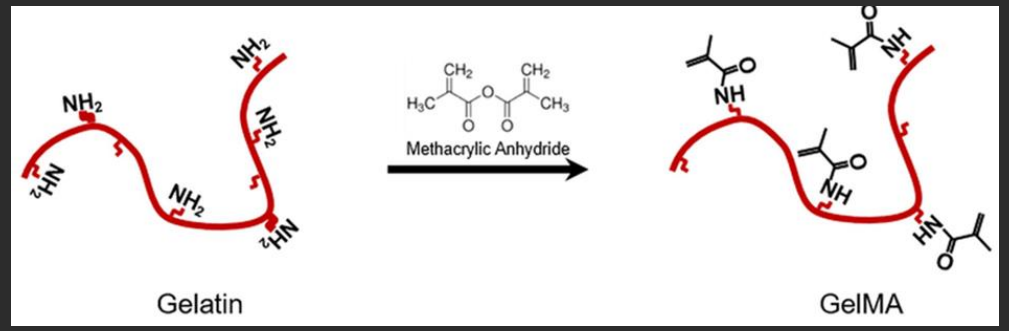
Materials

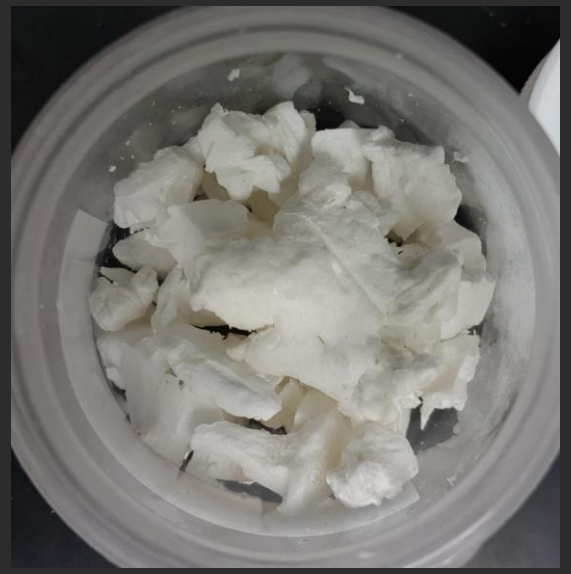
Polycaprolactone (PCL)

- Visco-elastic polyester
- FDA approved & biocompatible
- Slow degradation rate

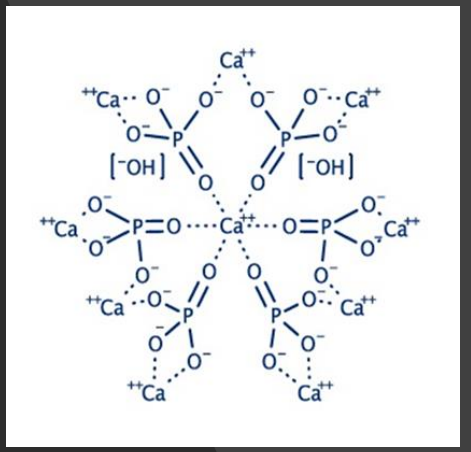


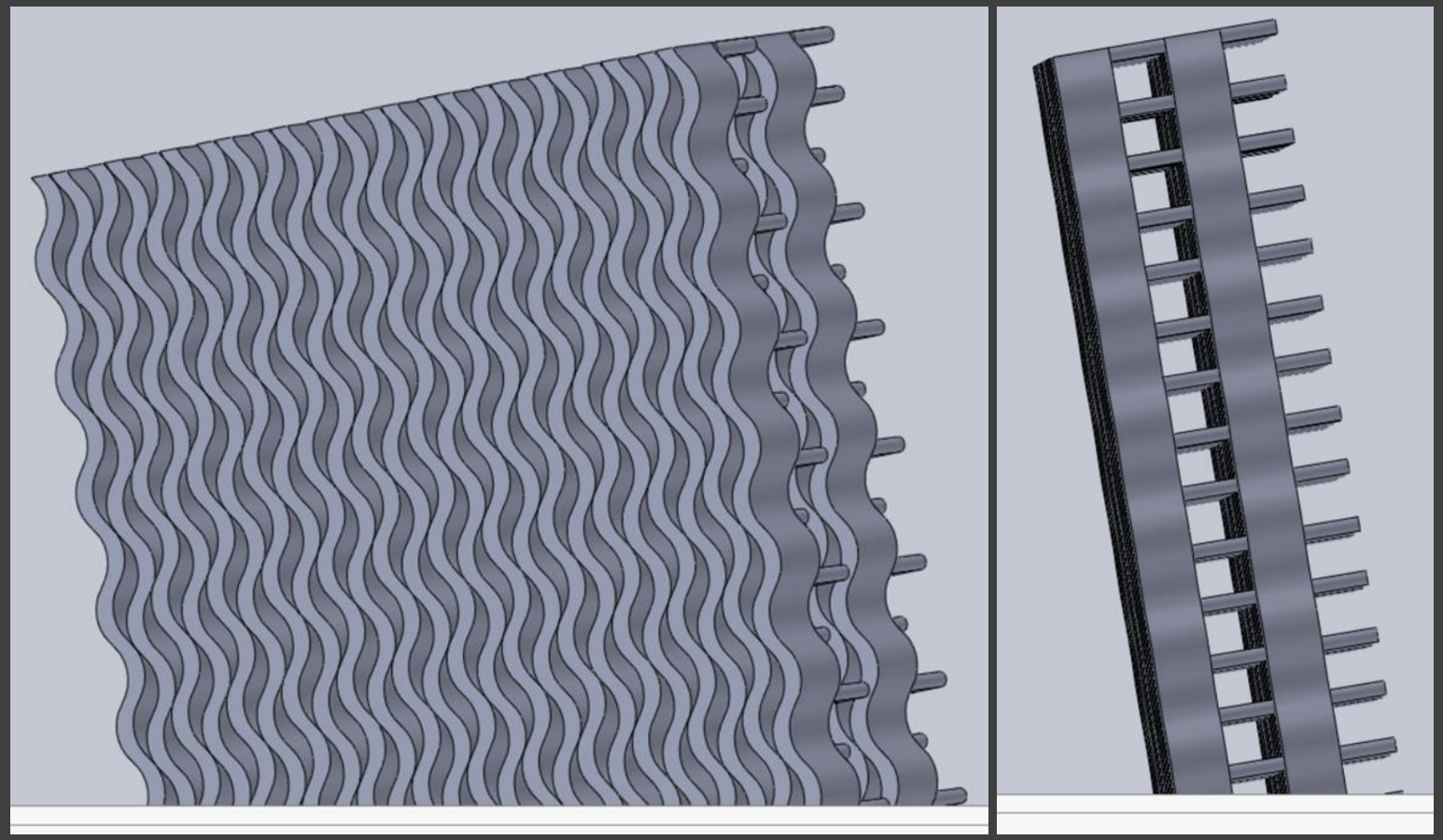
Gelatin methacryloyl (GelMA)





Hydroxyapatite (HA)



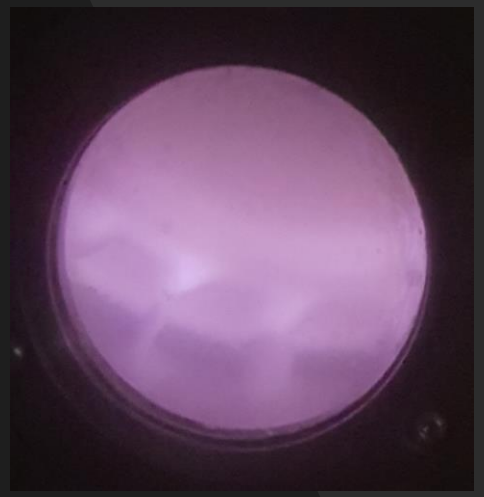


Scaffold Design: SolidWorks CAD Software

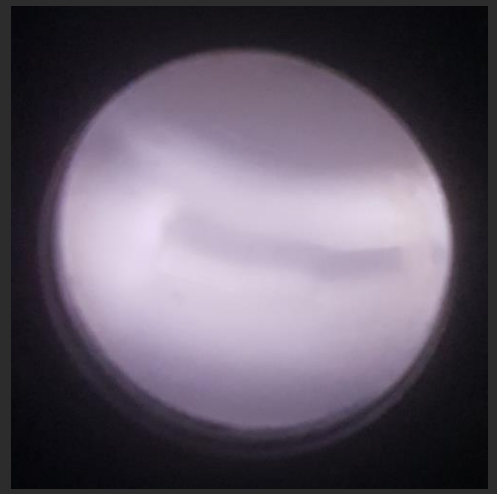
- Favorite design; couldn’t print due to resolution limitations

Plasma Modification

- Provides surface-level functional groups for improved interfacing with GelMA
- Low-Temperature Plasma (LTP) treatment
  - Non-equilibrium
  - Low-pressure
- 45 W radio frequency (RF), 15sccm, ~500mTorr

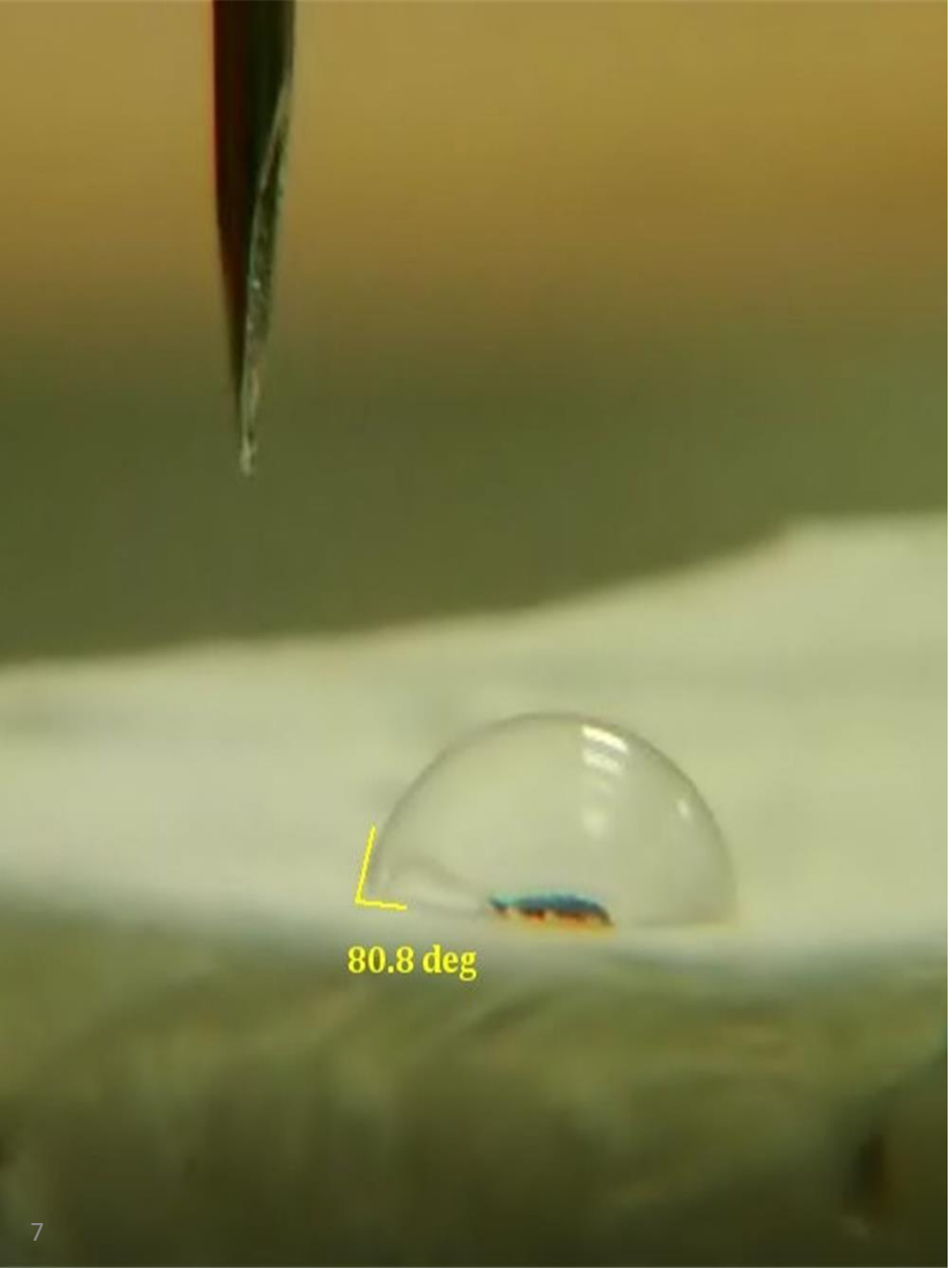


Nitrogen (N_2)



Oxygen (O_2)

Hydrogen (H_2)



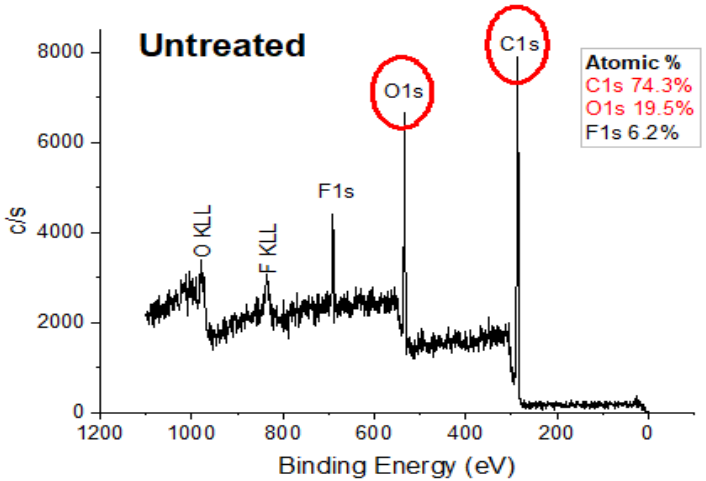
Water Contact Angle (Hydrophilicity)

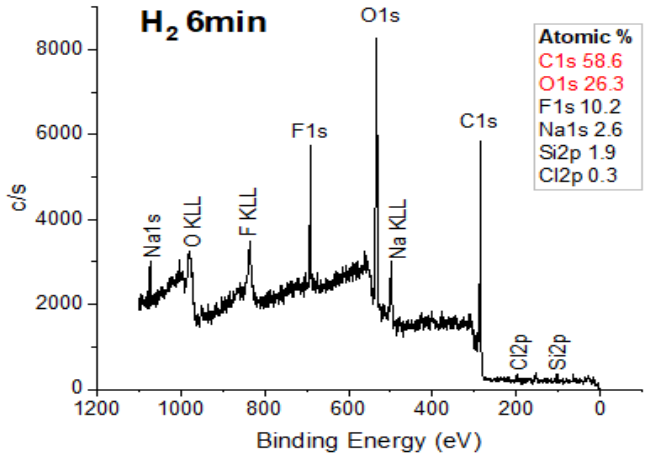
Time after drop:	1s	10s	30s
Plasma		**Contact Angle (°)**	
Untreated	80.8 ± 2.0	77.9 ± 2.0	72.2 ± 2.0
N 10s	27.8 ± 2.0	0	0
N 20s	0	0	0
N30s	0	0	0
O 10s	0	0	0
O 20s	0	0	0
O 30s	0	0	0
H 10s	82.2 ± 2.0	78.2 ± 2.0	76.1 ± 2.0
H 20s	0	0	0
H 30s	N/A	N/A	52.8 ± 2.0

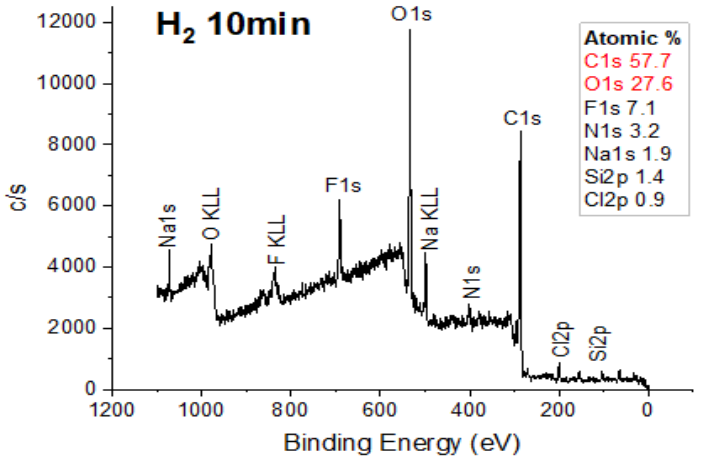
X-Ray Photoelectron Spectroscopy (XPS)

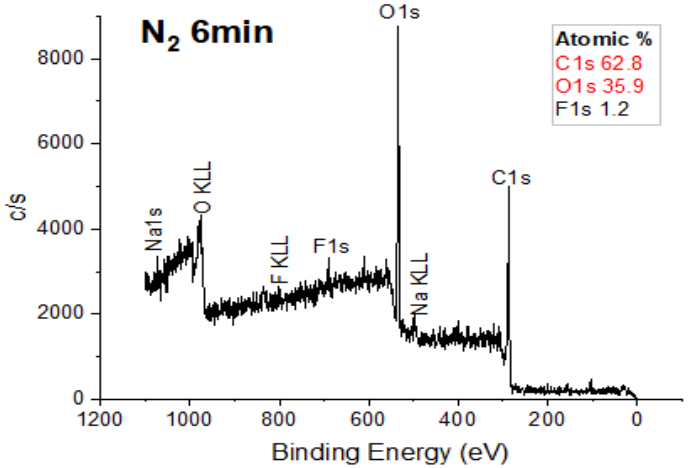
Oxygen treatment for 6+ minutes began to noticeably etch the surface

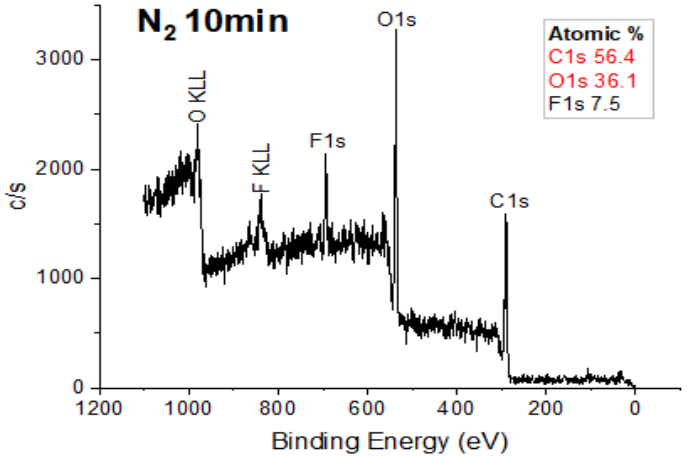
Plasma Treatment	Surface Element	Relative Percentage (%)
None (Control)	C	74.3
	O	**19.5**
Nitrogen 6min	C	62.8
	O	**35.9**
Nitrogen 10min	C	56.4
	O	**36.1**
Oxygen 2min	C	49.9
	O	**38.8**
Oxygen 3min	C	67.3
	O	**24**
	N	1.7
Oxygen 6min	C	53
	O	**27.9**
	O	26.3
Hydrogen 6min	C	58.6
	O	**26.3**
Hydrogen 10min	C	57.7
	O	**27.6**
	N	3.2

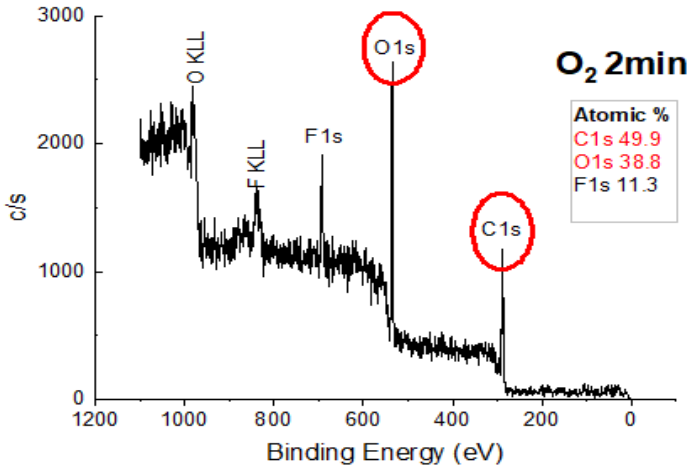

Untreated
Atomic %
C1s 74.3%
O1s 19.5%
F1s 6.2%
O1s
C1s
F1s
O KLL
F KLL
c/s
Binding Energy (eV)

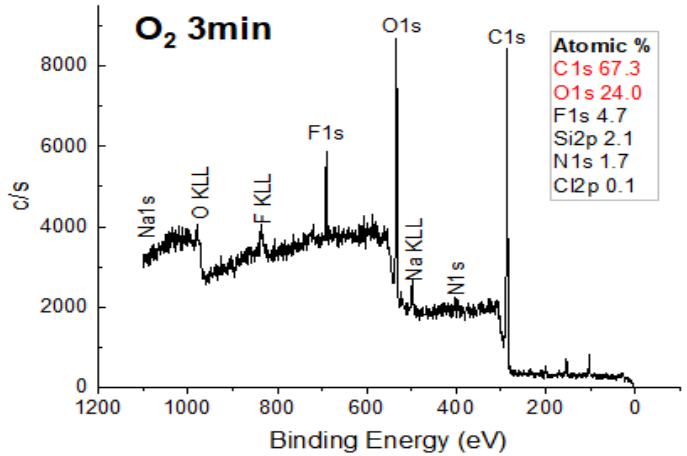

H2 6min
Atomic %
C1s 58.6
O1s 26.3
F1s 10.2
Na1s 2.6
Si2p 1.9
Cl2p 0.3
O1s
F1s
C1s
Na1s
O KLL
F KLL
Na KLL
Cl2p
Si2p
c/s
Binding Energy (eV)

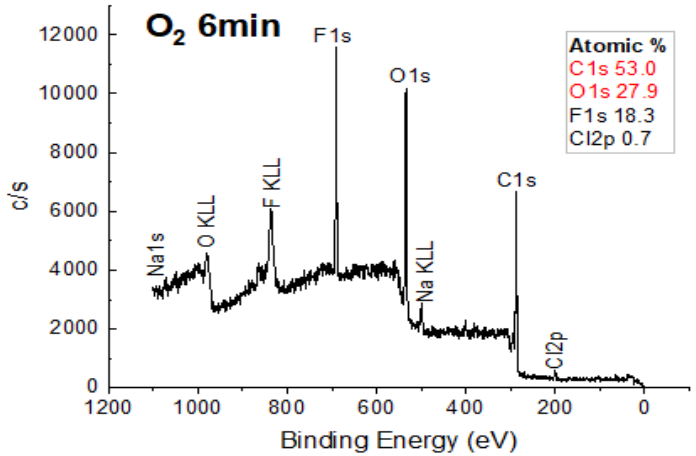

H2 10min
Atomic %
C1s 57.7
O1s 27.6
F1s 7.1
N1s 3.2
Na1s 1.9
Si2p 1.4
Cl2p 0.9
O1s
F1s
C1s
Na1s
O KLL
F KLL
Na KLL
N1s
Cl2p
Si2p
c/s
Binding Energy (eV)


N2 6min
Atomic %
C1s 62.8
O1s 35.9
F1s 1.2
O1s
C1s
Na1s
O KLL
F KLL
F1s
Na KLL
c/s
Binding Energy (eV)


N2 10min
Atomic %
C1s 56.4
O1s 36.1
F1s 7.5
O1s
O KLL
F KLL
F1s
C1s
c/s
Binding Energy (eV)

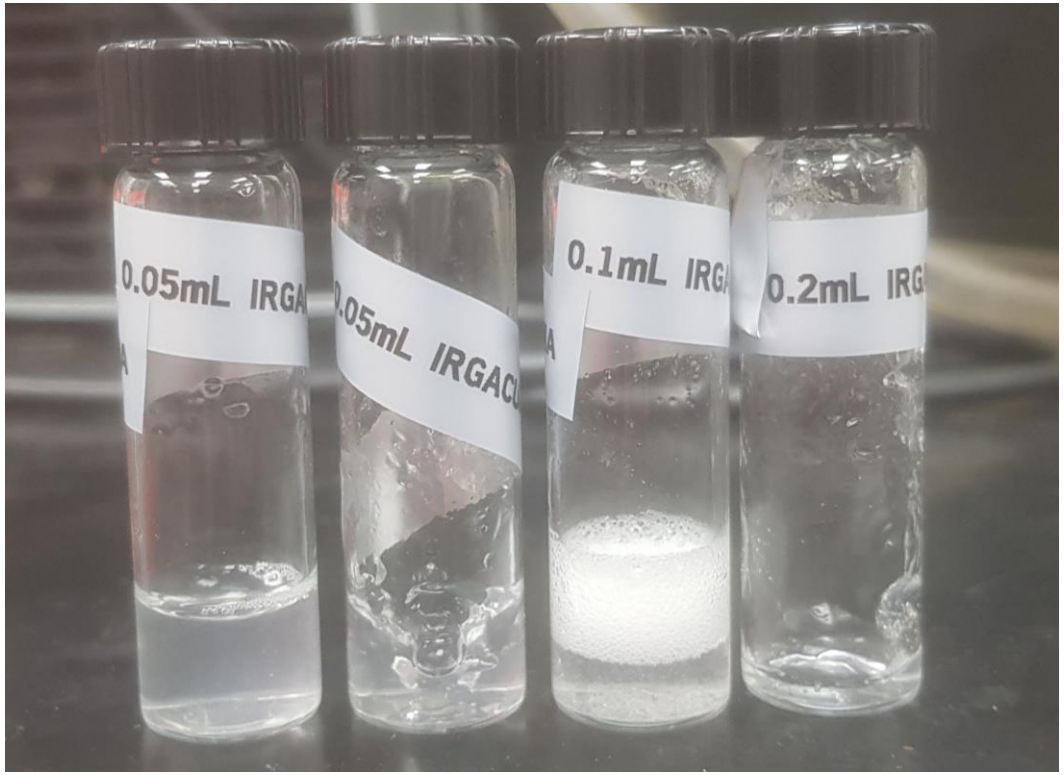

O2 2min
Atomic %
C1s 49.9
O1s 38.8
F1s 11.3
O1s
O KLL
F KLL
F1s
C1s
c/s
Binding Energy (eV)


O2 3min
Atomic %
C1s 67.3
O1s 24.0
F1s 4.7
Si2p 2.1
N1s 1.7
Cl2p 0.1
O1s
C1s
F1s
Na1s
O KLL
F KLL
Na KLL
N1s
c/s
Binding Energy (eV)


O2 6min
Atomic %
C1s 53.0
O1s 27.9
F1s 18.3
Cl2p 0.7
F1s
O1s
C1s
Na1s
O KLL
F KLL
Na KLL
Cl2p
c/s
Binding Energy (eV)

Fabrication/Optimization of Hydrogel

- GelMA and Irgacure 2959 (photoinitiator) were separately mixed in dPBS
- Irgacure was added to GelMA mixture in varying concentrations
- Mixture was photopolymerized with UV light (365nm) for different amounts of time
- Optimal combination was 1.5% photoinitiator, crosslinked for 3 minutes
- For GelMA-HA, nano hydroxyapatite (HA) powder was added at a 5% w/v concentration



Optimization of Hydrogels via Rheology

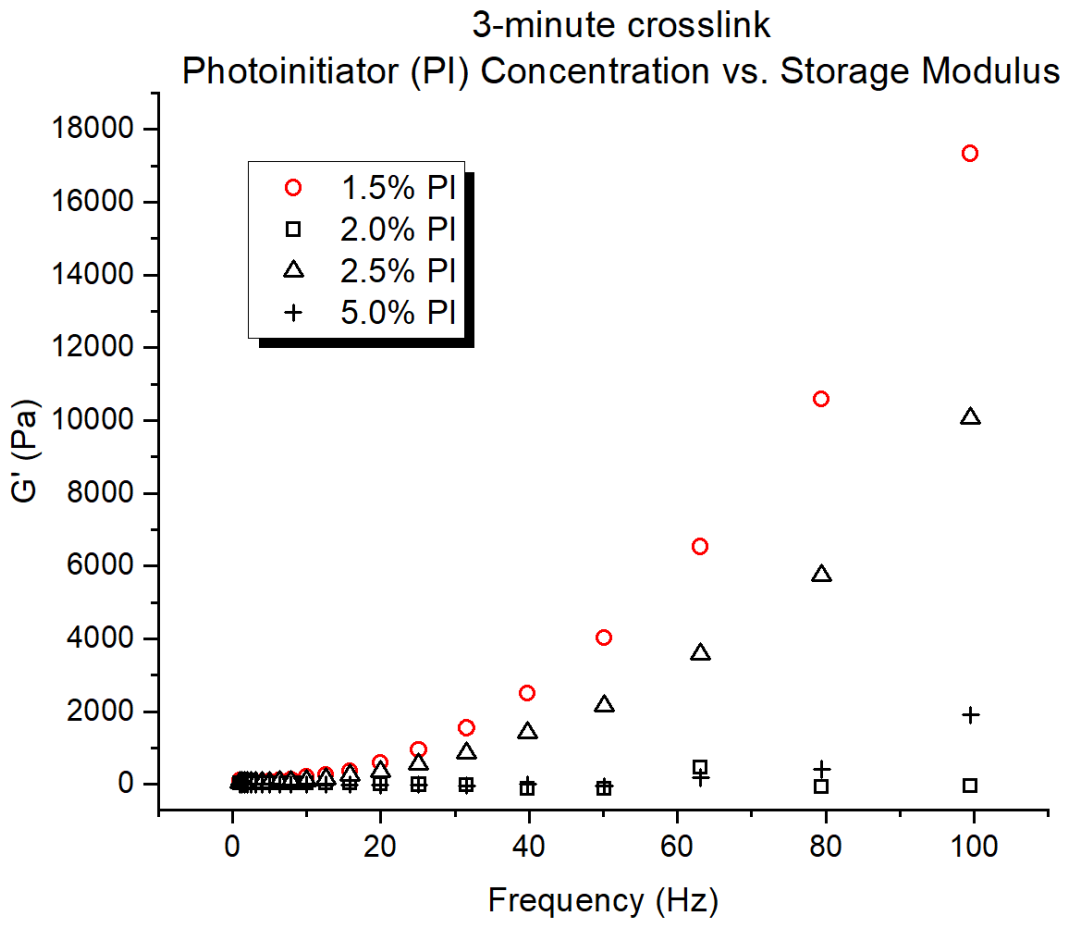
3-minute crosslink

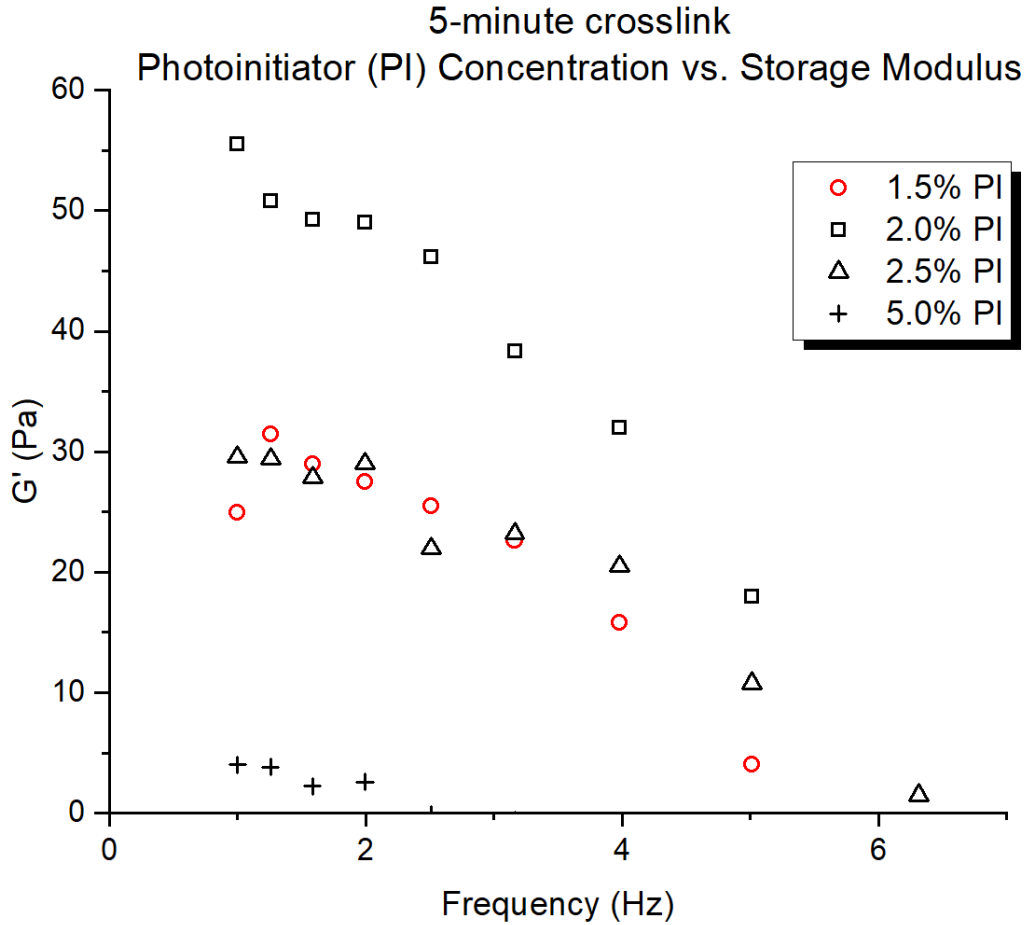
PI (% w/v)	Max G' (Pa)
1.50%	17340
2.00%	464.5
2.50%	10070
5.00%	1908

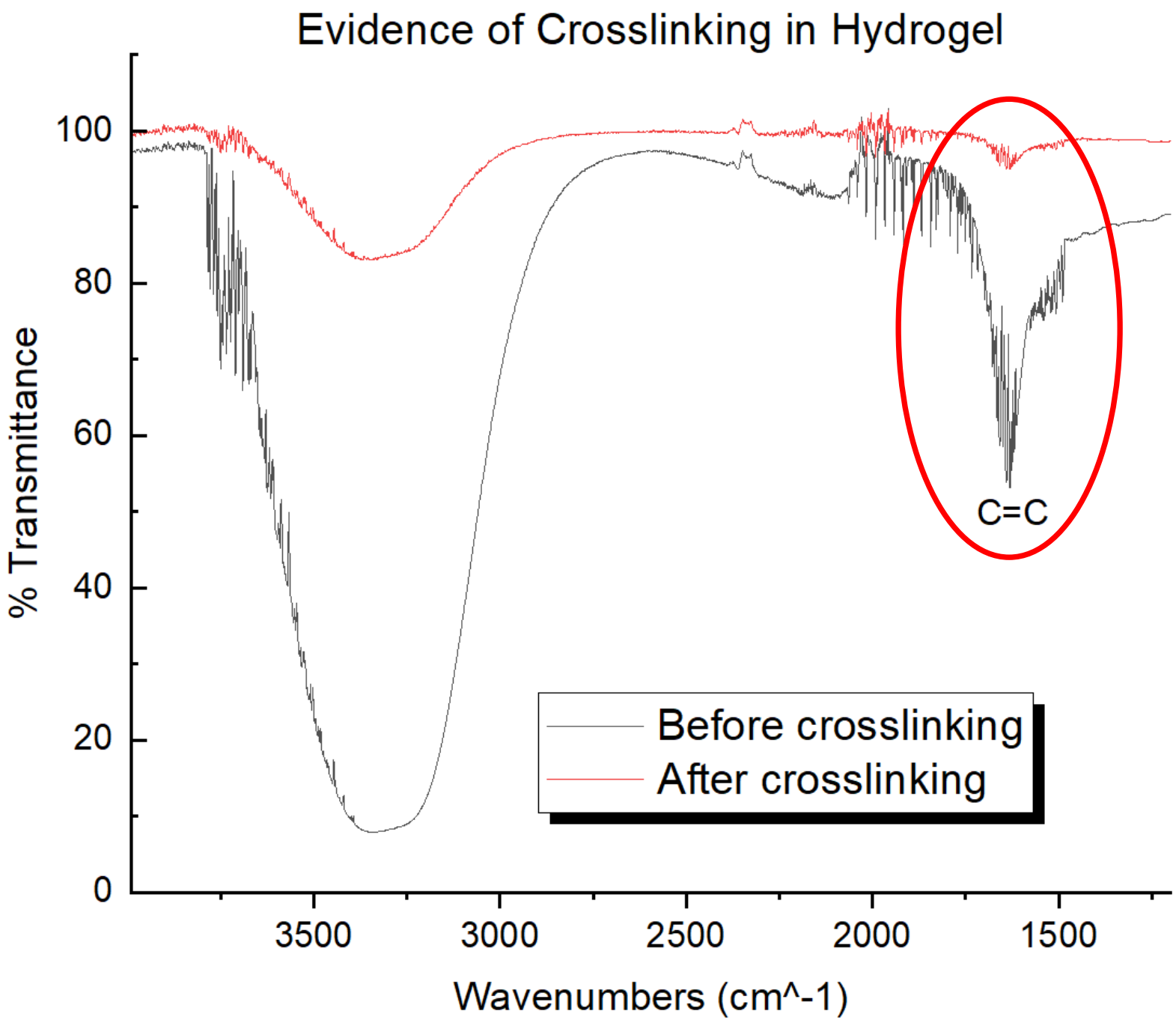
5-minute crosslink

PI (% w/v)	Max G' (Pa)
1.50%	31.46
2.00%	55.53
2.50%	29.58
5.00%	4.014

Storage Modulus



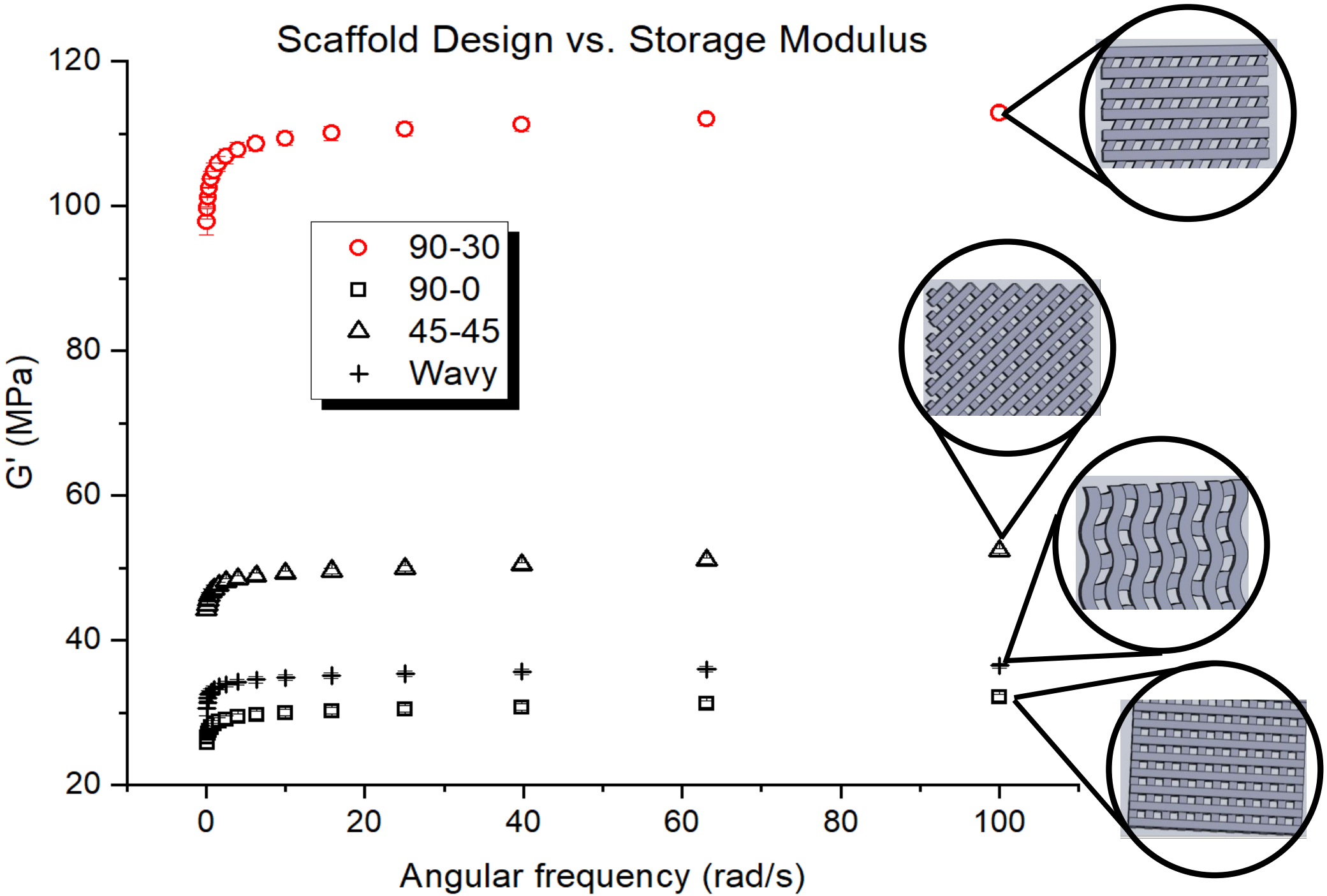


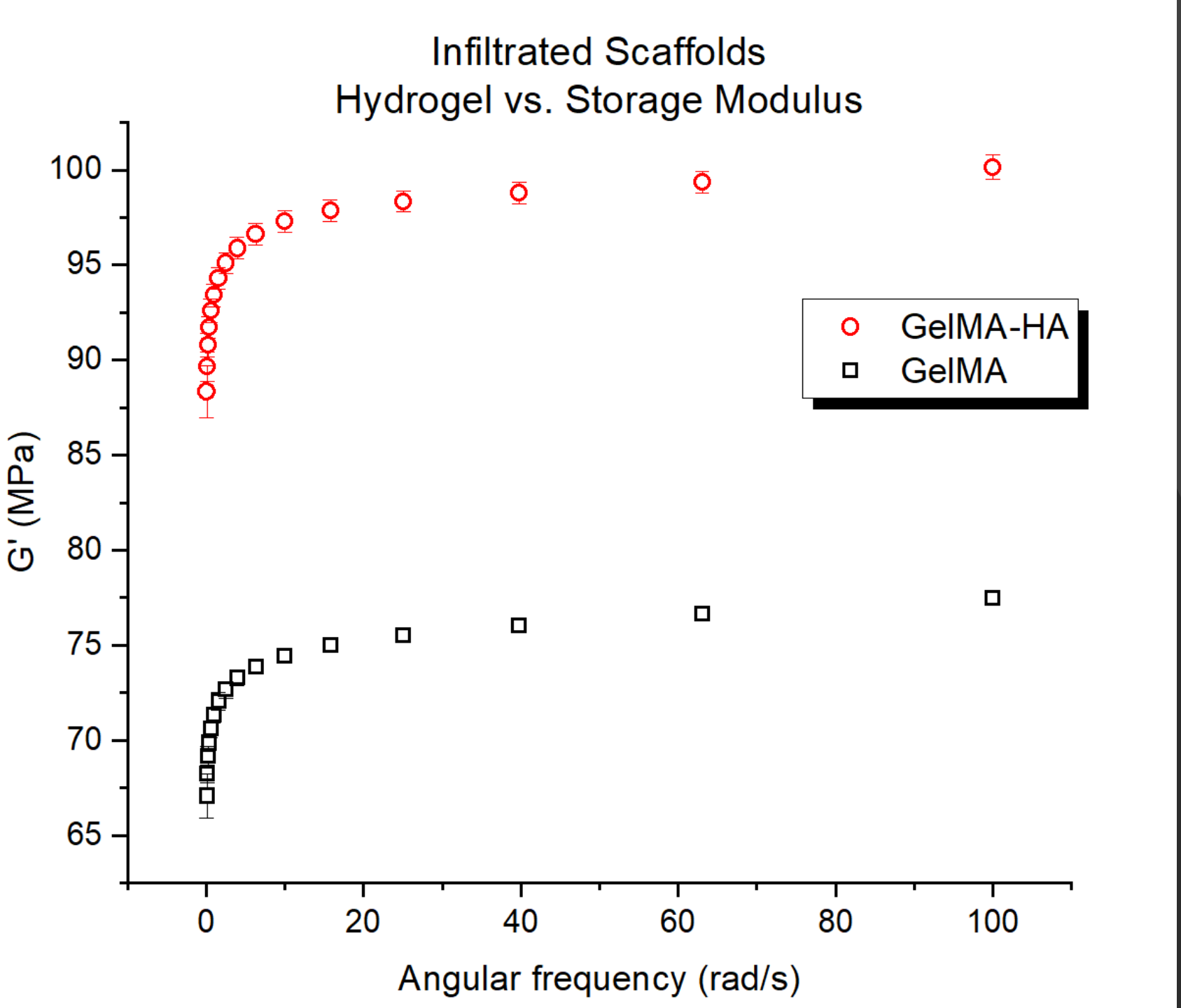

Evidence of Crosslinking in Hydrogel
100
80
60
40
20
0
% Transmittance
3500
3000
2500
2000
1500
Wavenumbers (cm^-1)
C=C
Before crosslinking
After crosslinking

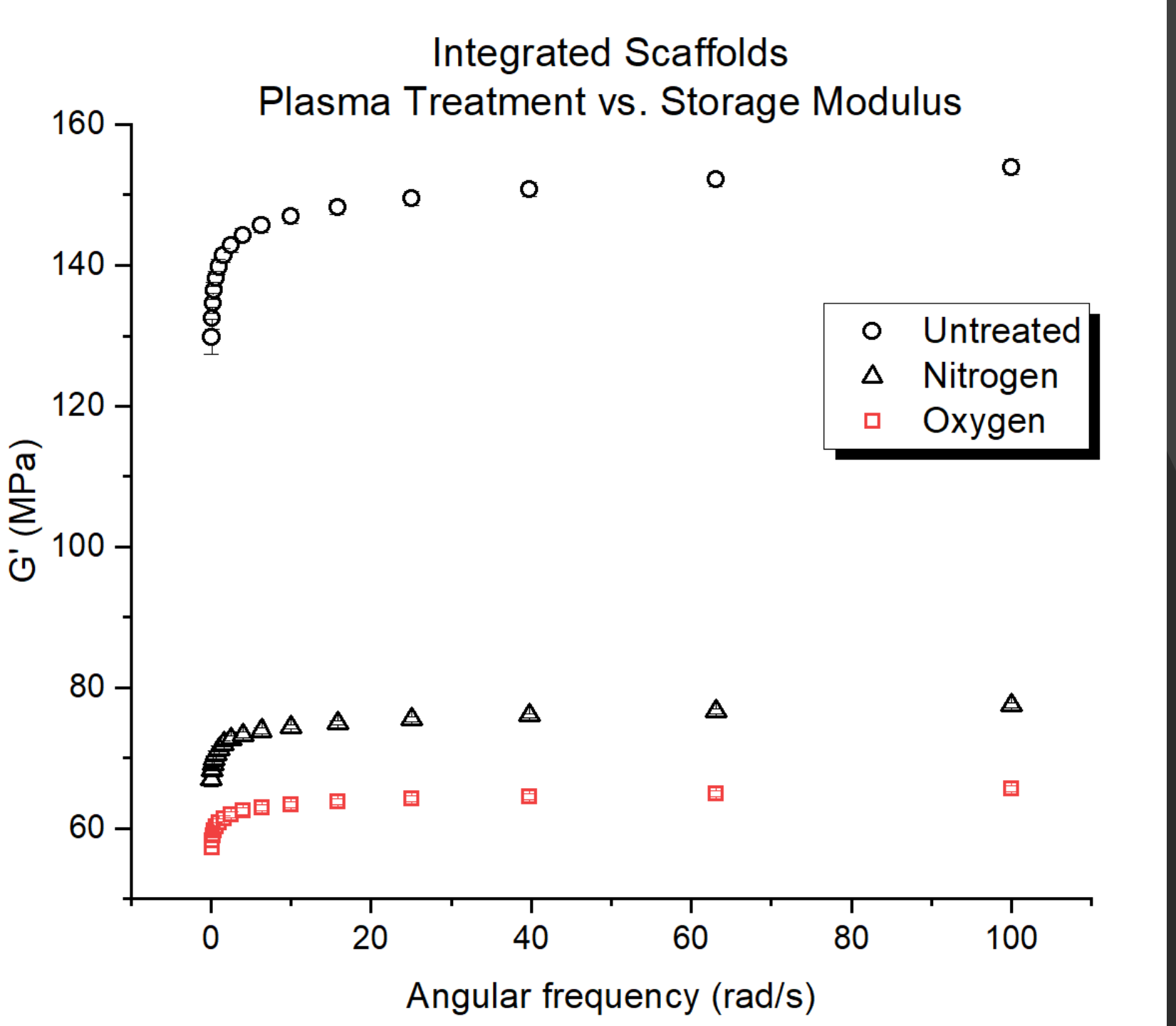
Hydrogel Infiltration

- White is GelMA-HA composite
- Poured GelMA/GelMA-HA solution over scaffold
- De-gassed in a vacuum chamber for infiltration into pores
- Crosslinked with 365nm UV light
- Removed scaffolds with tweezers

Mechanical Strength Testing (Torsion)

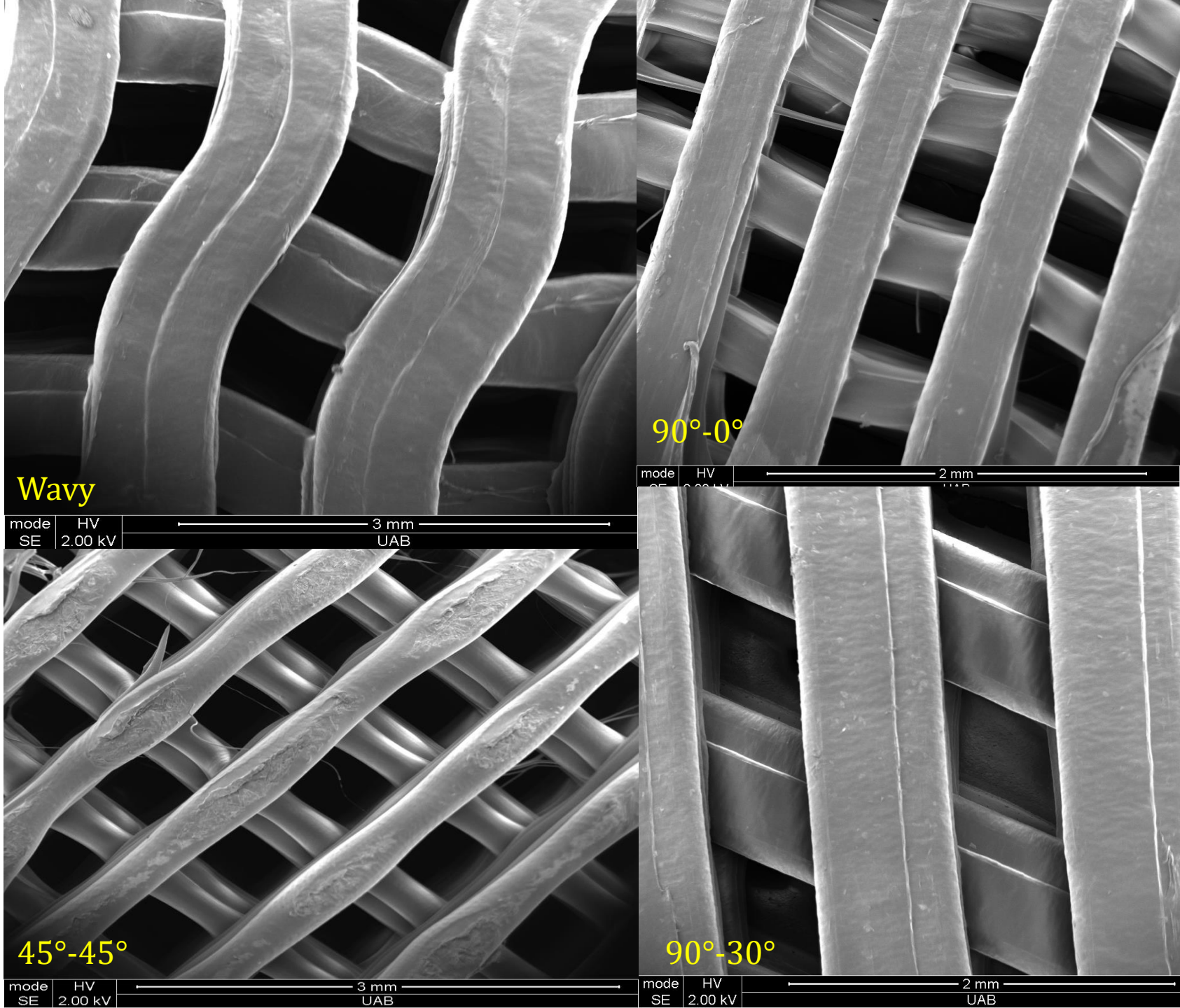

Scaffold Design vs. Storage Modulus
G' (MPa)
120
100
80
60
40
20
0
20
40
60
80
100
Angular frequency (rad/s)
90-30
90-0
45-45
Wavy

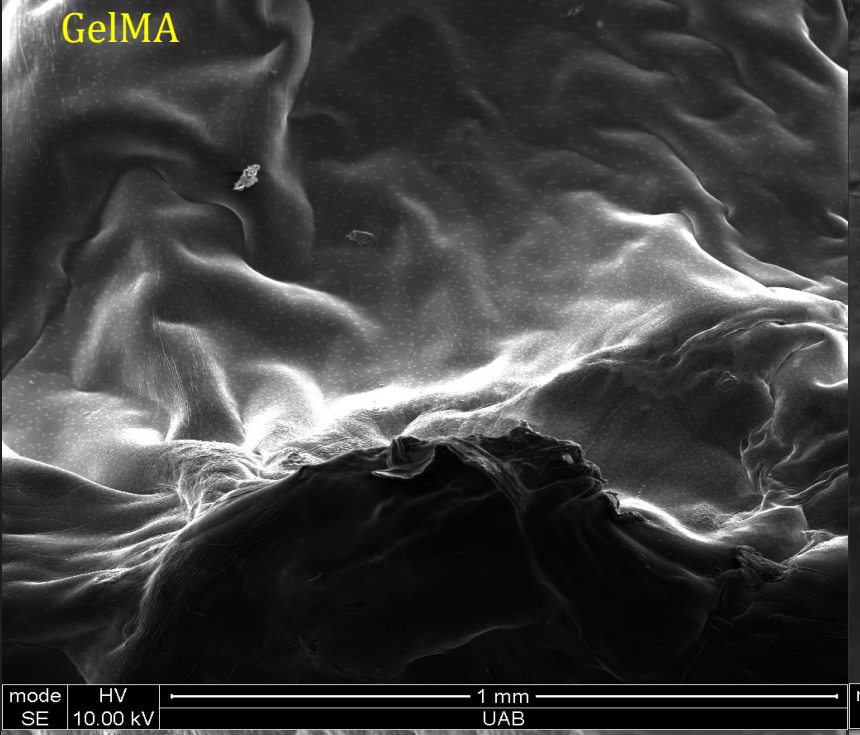

Infiltrated Scaffolds
Hydrogel vs. Storage Modulus
G' (MPa)
100
95
90
85
80
75
70
65
0
20
40
60
80
100
Angular frequency (rad/s)
GelMA-HA
GelMA

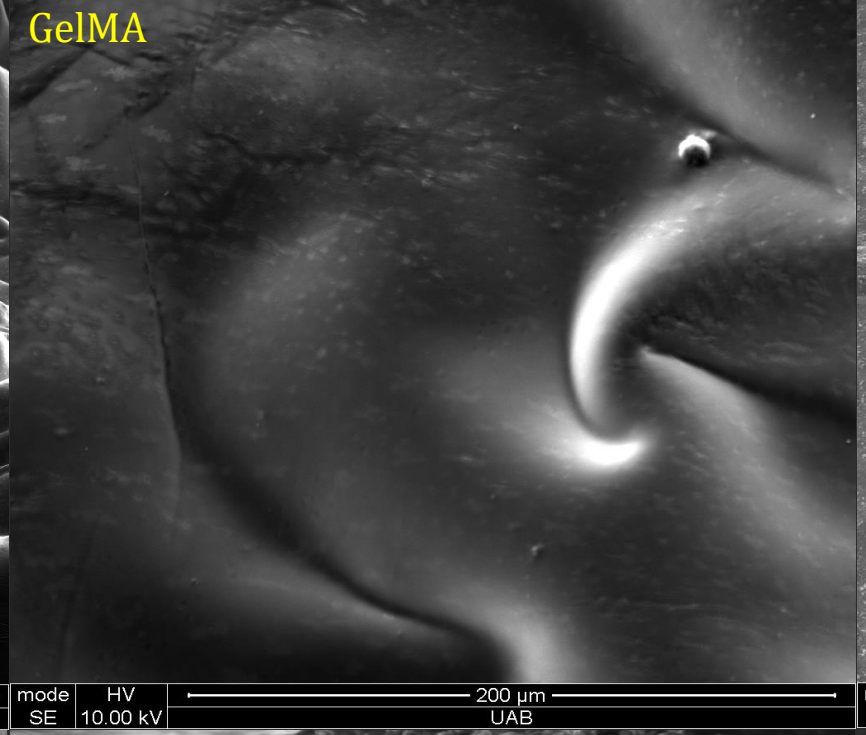

Integrated Scaffolds
Plasma Treatment vs. Storage Modulus
G' (MPa)
160
140
120
100
80
60
0
20
40
60
80
100
Angular frequency (rad/s)
Untreated
Nitrogen
Oxygen

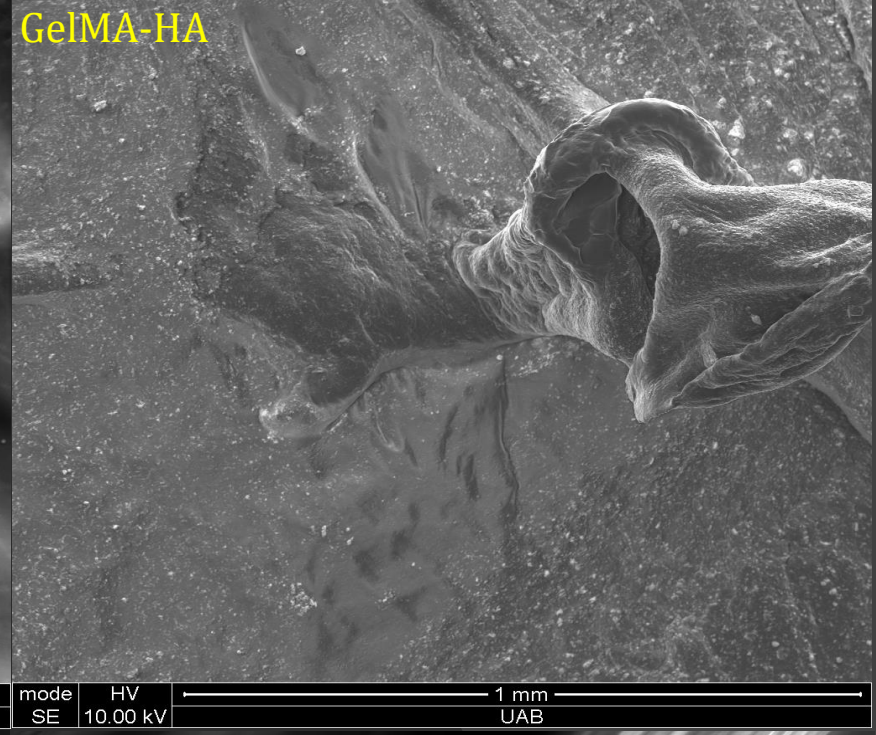
Scanning Electron Microscopy (SEM)

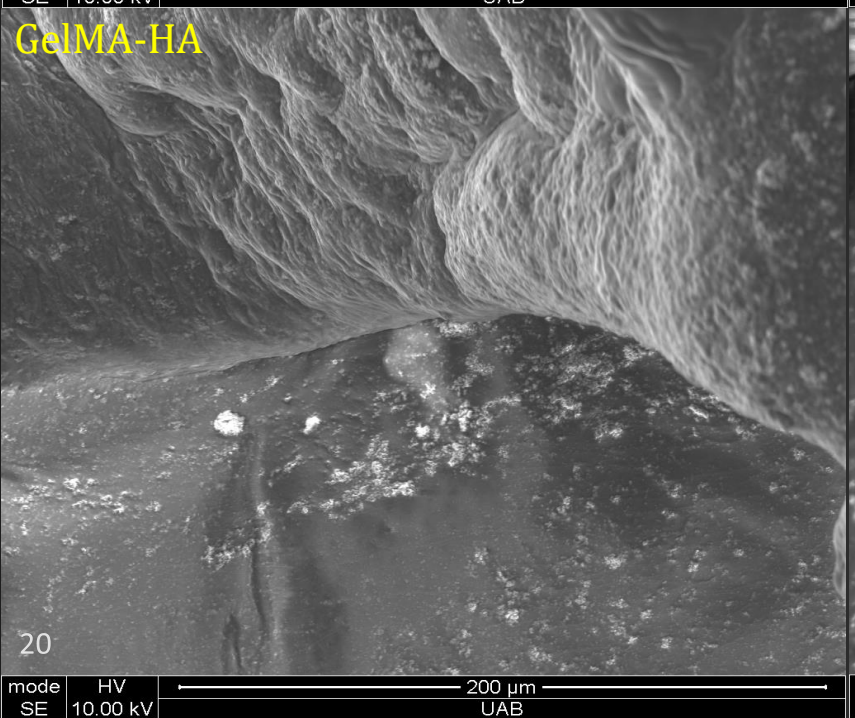
Scaffolds of varying designs and pore structure

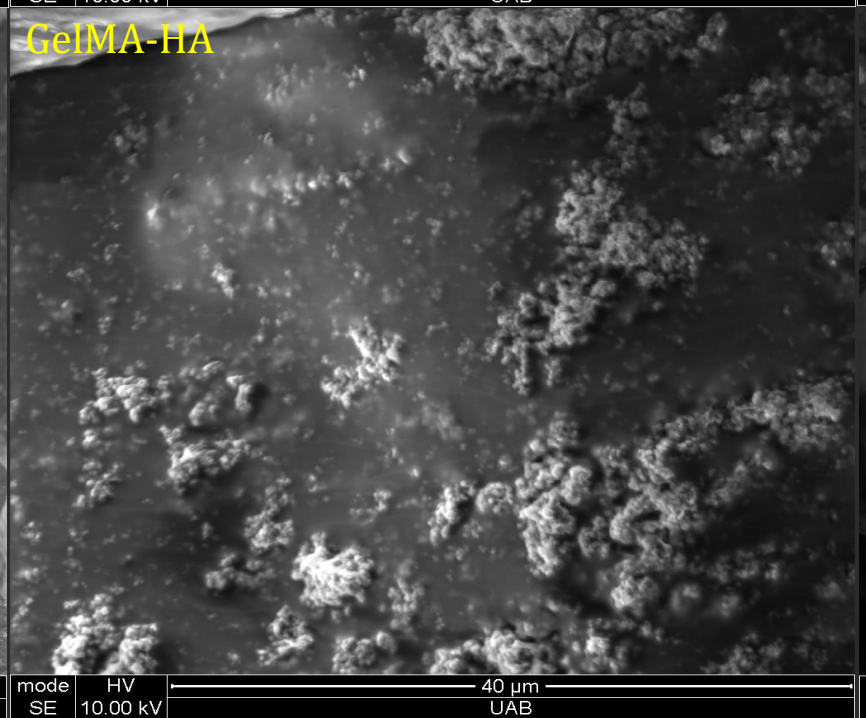


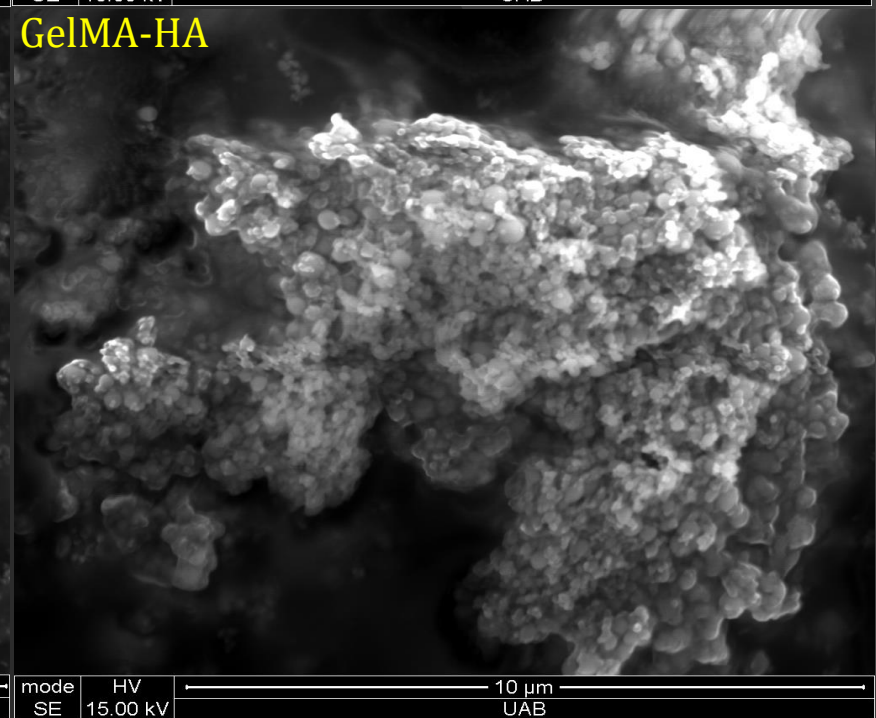

GelMA
mode SE
HV 10.00 kV
1 mm
UAB


GelMA
mode SE
HV 10.00 kV
200 µm
UAB

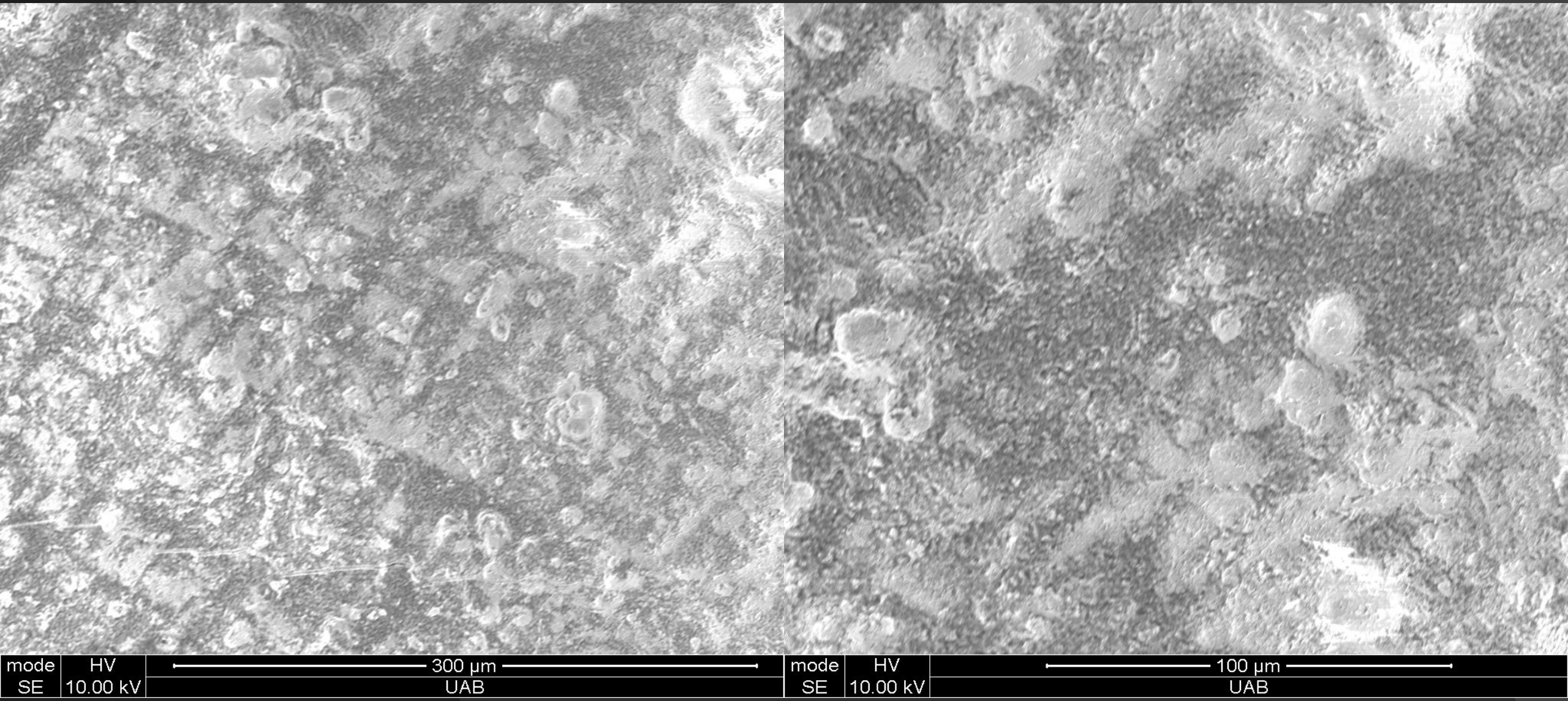

GelMA-HA
mode SE
HV 10.00 kV
1 mm
UAB

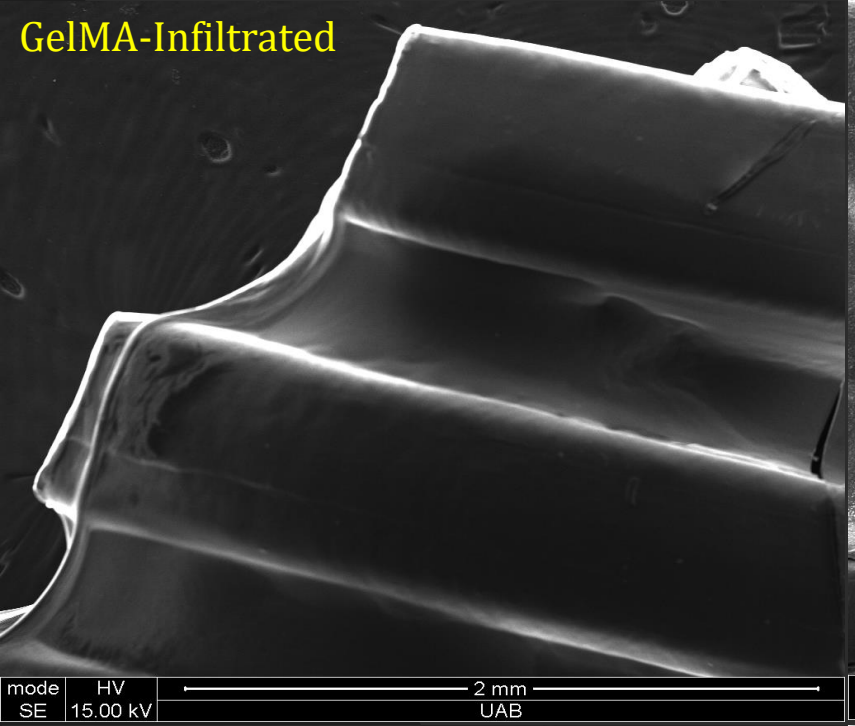

GelMA-HA
mode SE
HV 10.00 kV
200 µm
UAB

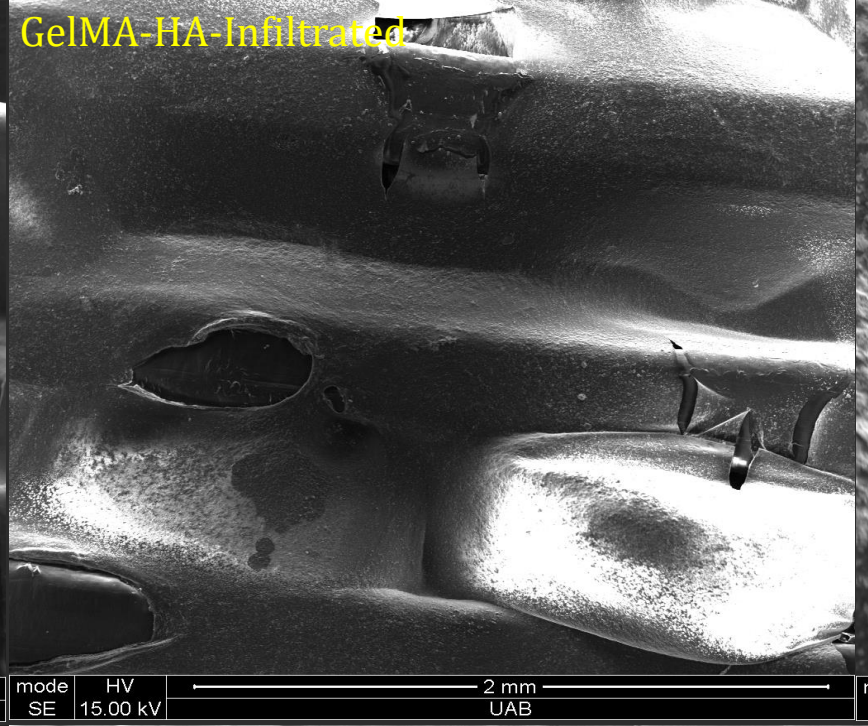

GelMA-HA
mode SE
HV 10.00 kV
40 µm
UAB

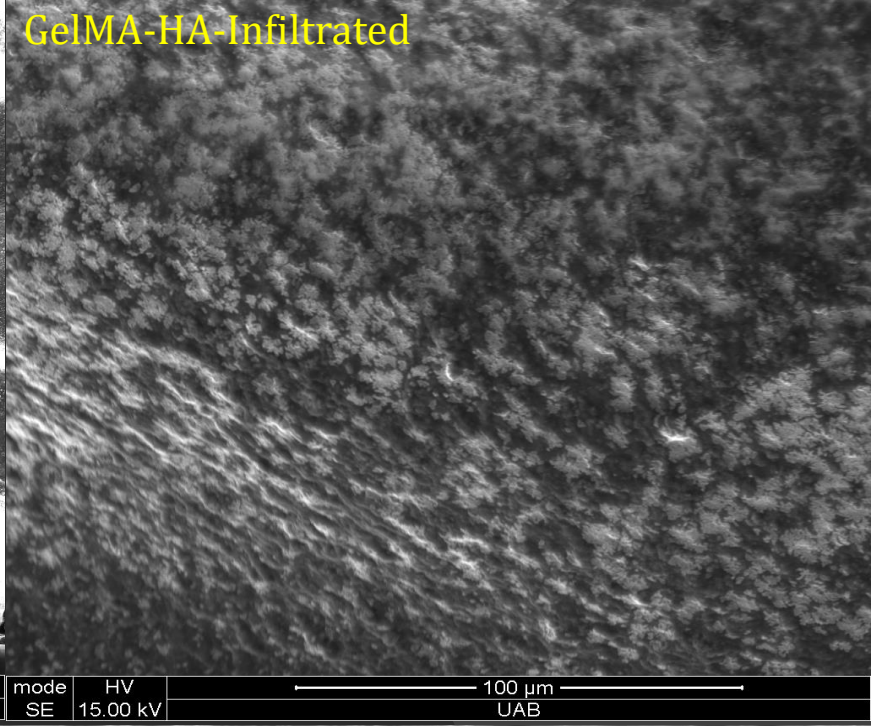

GelMA-HA
mode SE
HV 15.00 kV
10 µm
UAB

Hydroxyapatite

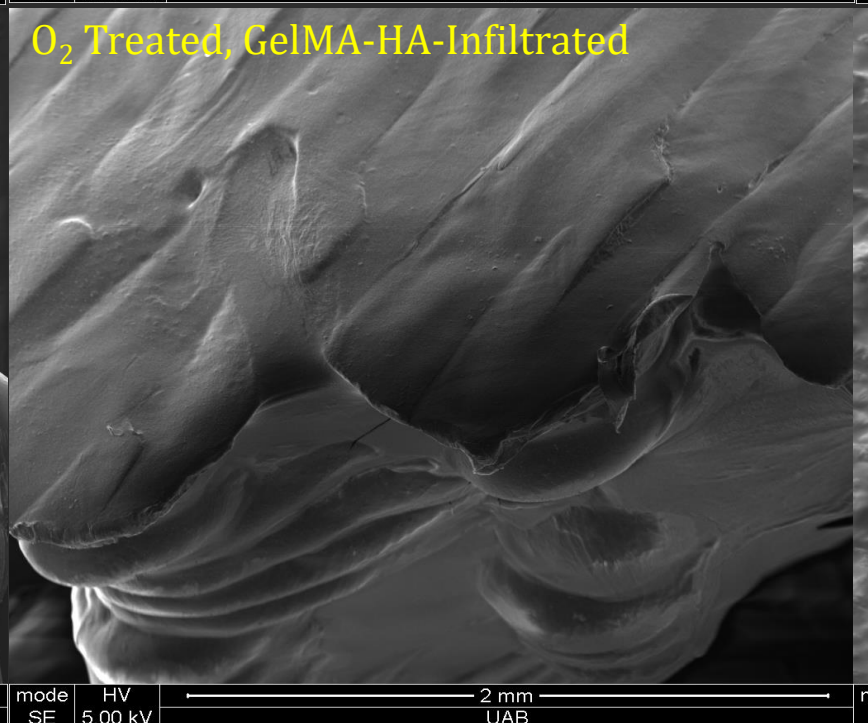


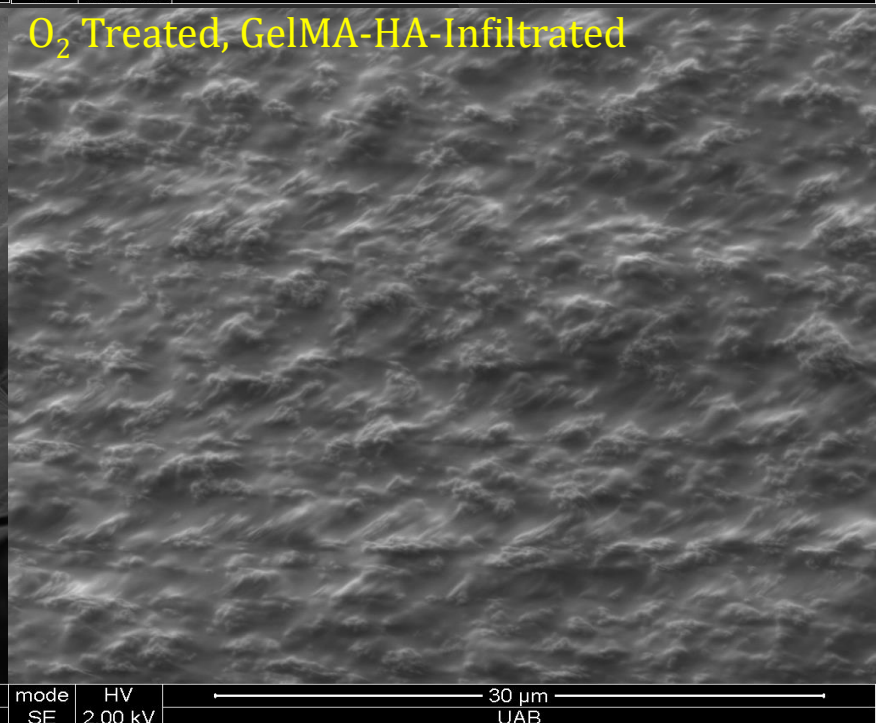

GelMA-Infiltrated
mode SE
HV 15.00 kV
2 mm
UAB

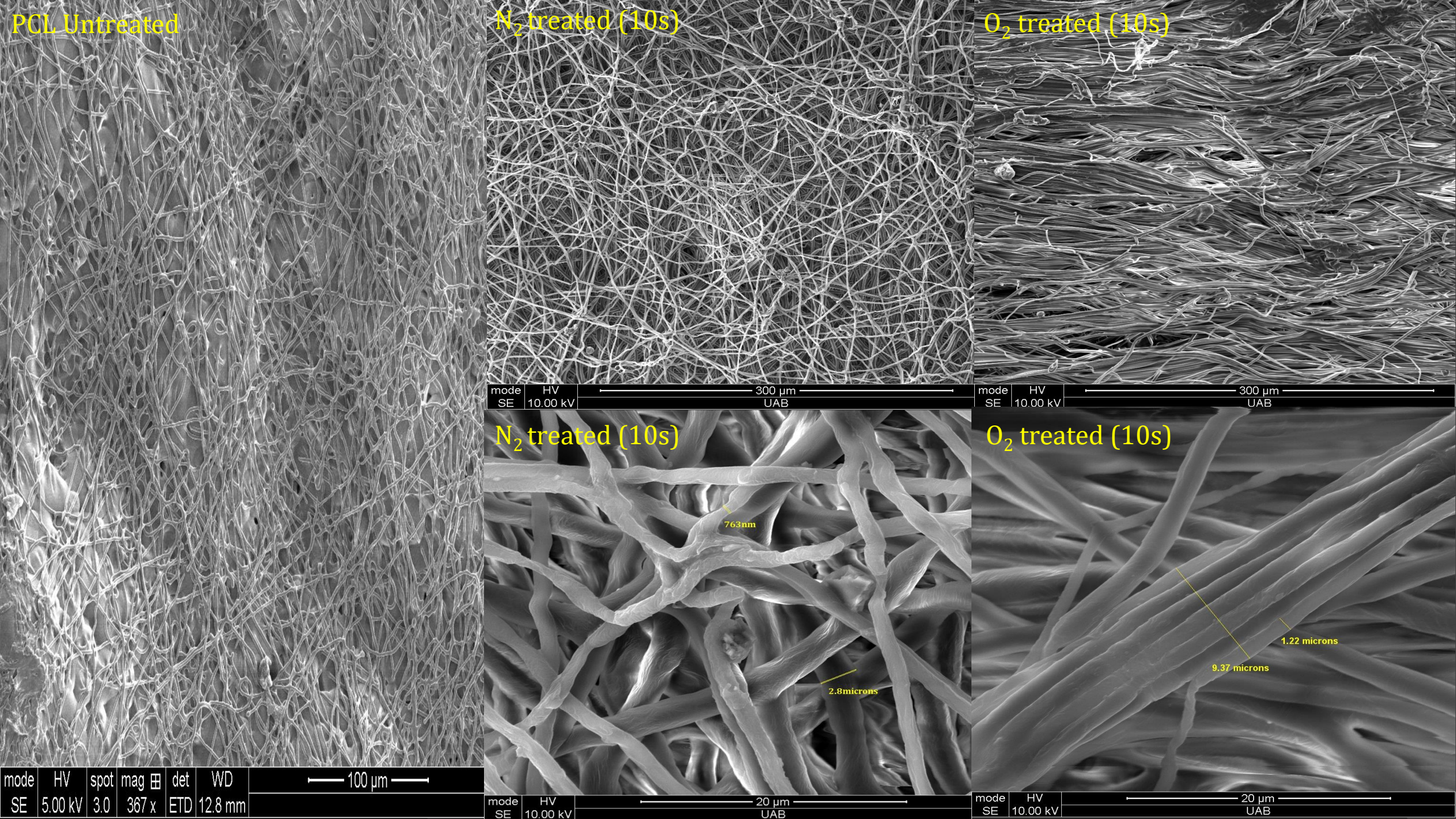

GelMA-HA-Infiltrated
mode SE
HV 15.00 kV
2 mm
UAB

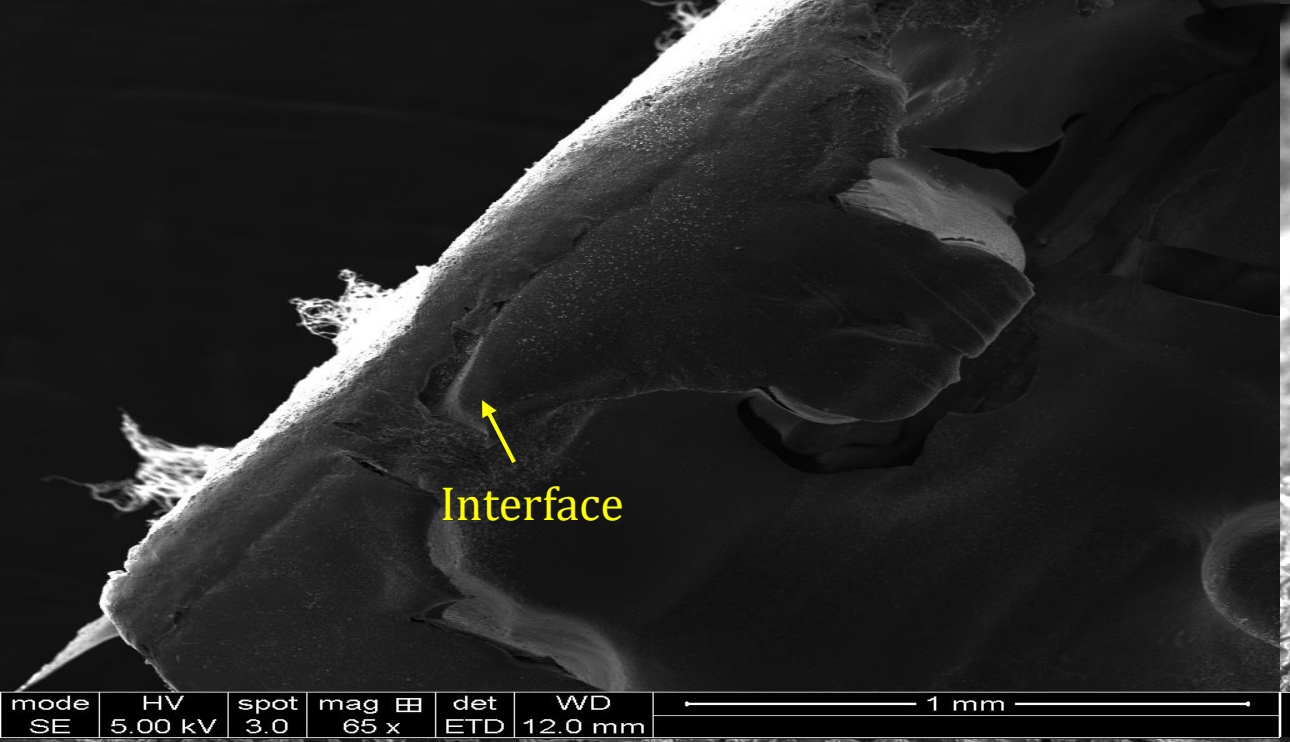

GelMA-HA-Infiltrated
mode SE
HV 15.00 kV
100 µm
UAB

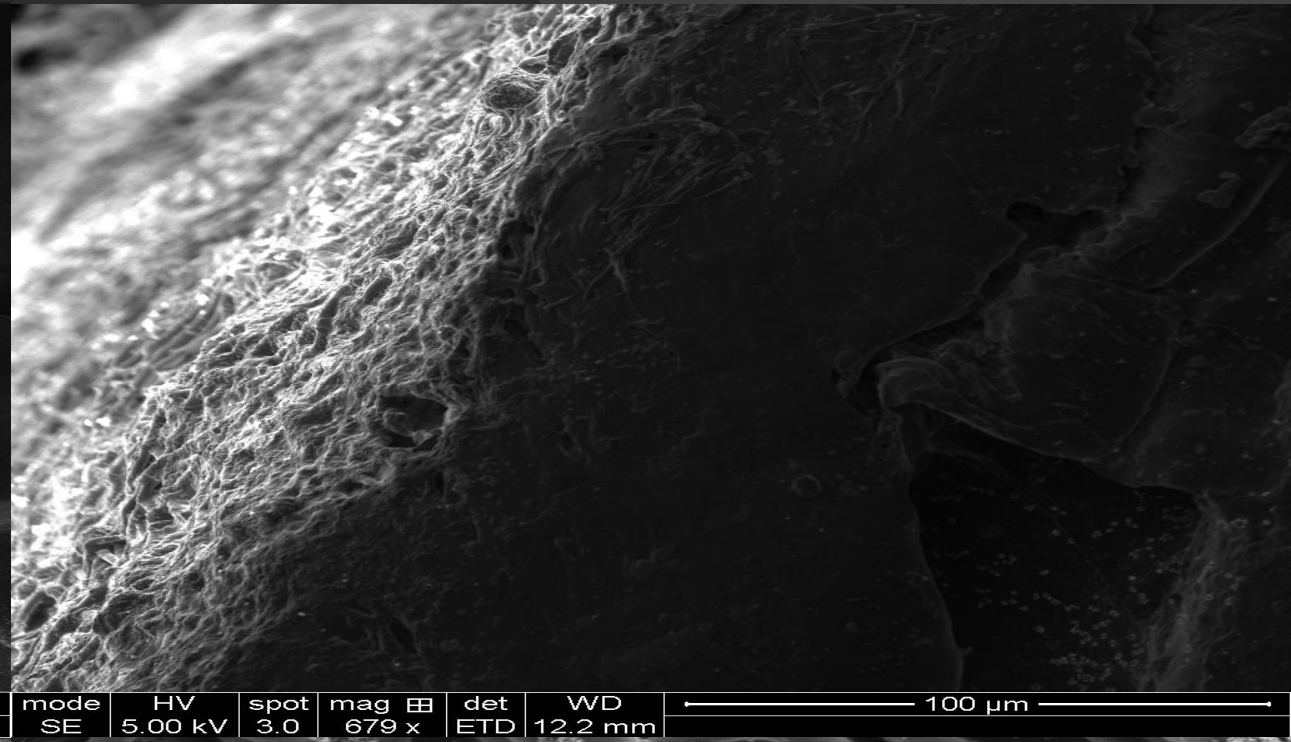

O_2 Treated, GelMA-Infiltrated
mode SE
HV 10.00 kV
2 mm
UAB

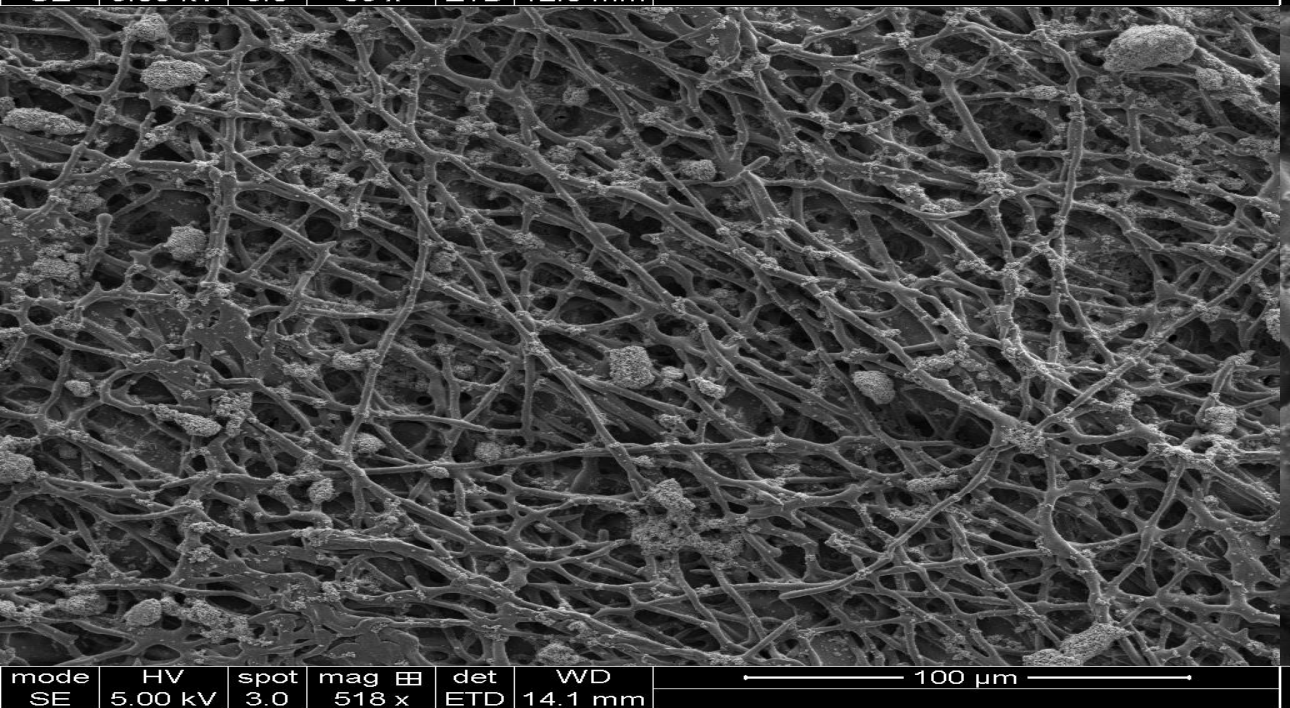

O_2 Treated, GelMA-HA-Infiltrated
mode SE
HV 5.00 kV
2 mm
UAB

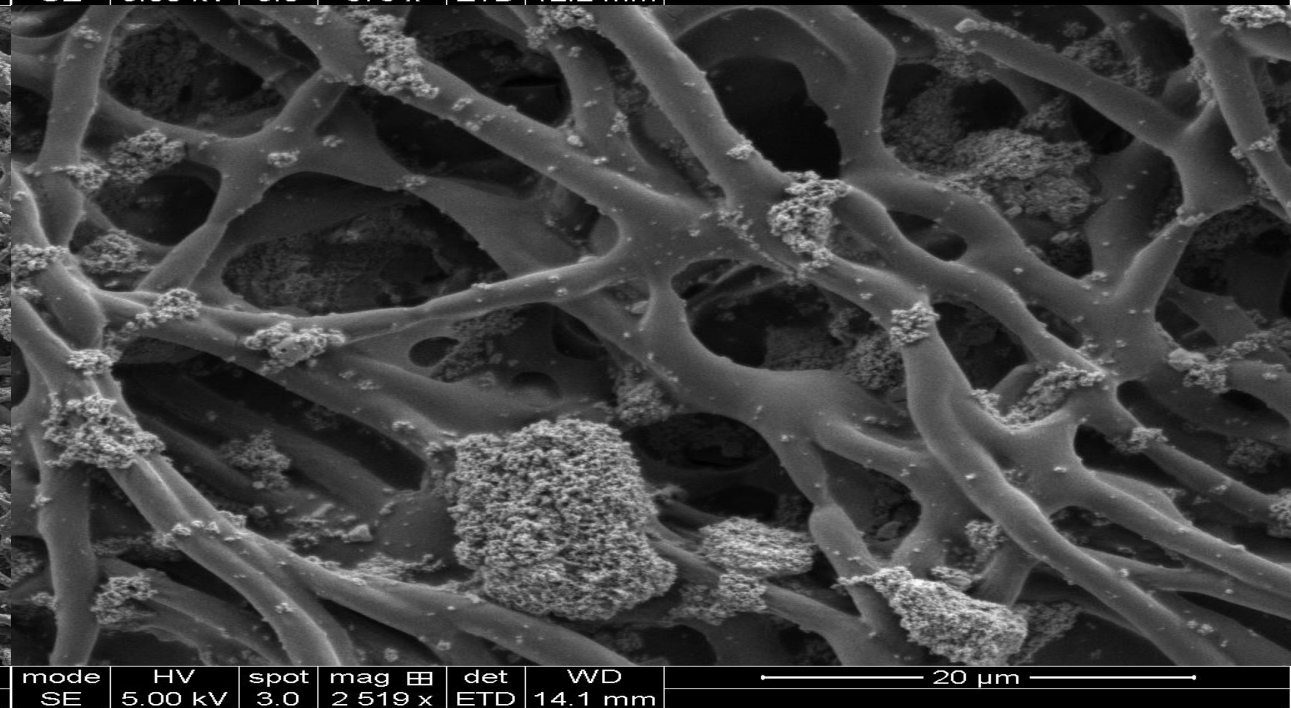

O_2 Treated, GelMA-HA-Infiltrated
mode SE
HV 2.00 kV
30 µm
UAB

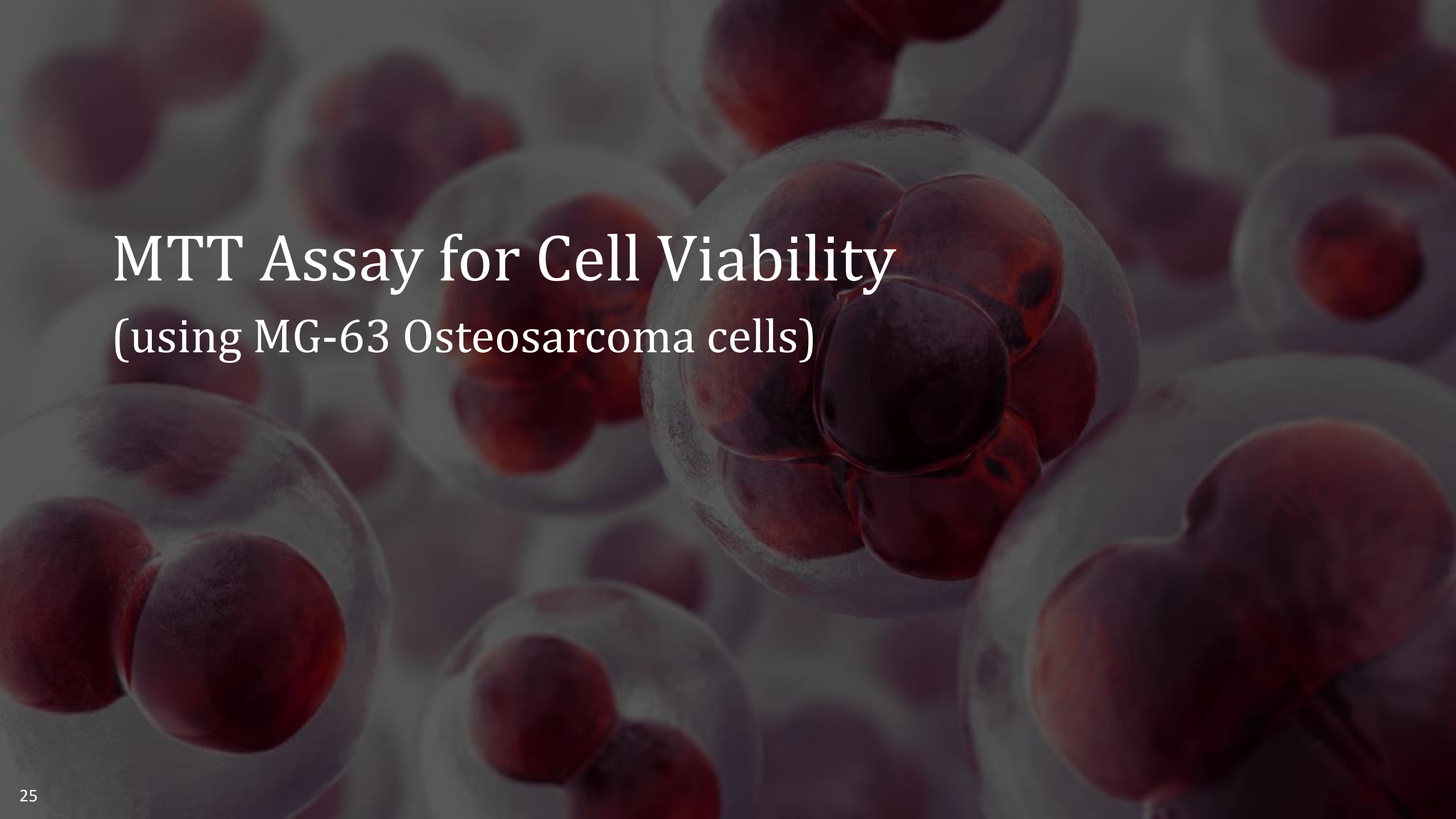

PCL Untreated
mode SE
HV 5.00 kV
spot 3.0
mag 367 x
det ETD
WD 12.8 mm
100 µm
N2 treated (10s)
mode SE
HV 10.00 kV
300 µm
UAB
O2 treated (10s)
mode SE
HV 10.00 kV
300 µm
UAB
N2 treated (10s)
763nm
2.8microns
mode SE
HV 10.00 kV
20 µm
UAB
O2 treated (10s)
1.22 microns
9.37 microns
mode SE
HV 10.00 kV
20 µm
UAB


Interface
mode SE
HV 5.00 kV
spot 3.0
mag 65 x
det ETD
WD 12.0 mm
1 mm


mode SE
HV 5.00 kV
spot 3.0
mag 679 x
det ETD
WD 12.2 mm
100 µm


mode SE
HV 5.00 kV
spot 3.0
mag 518 x
det ETD
WD 14.1 mm
100 µm

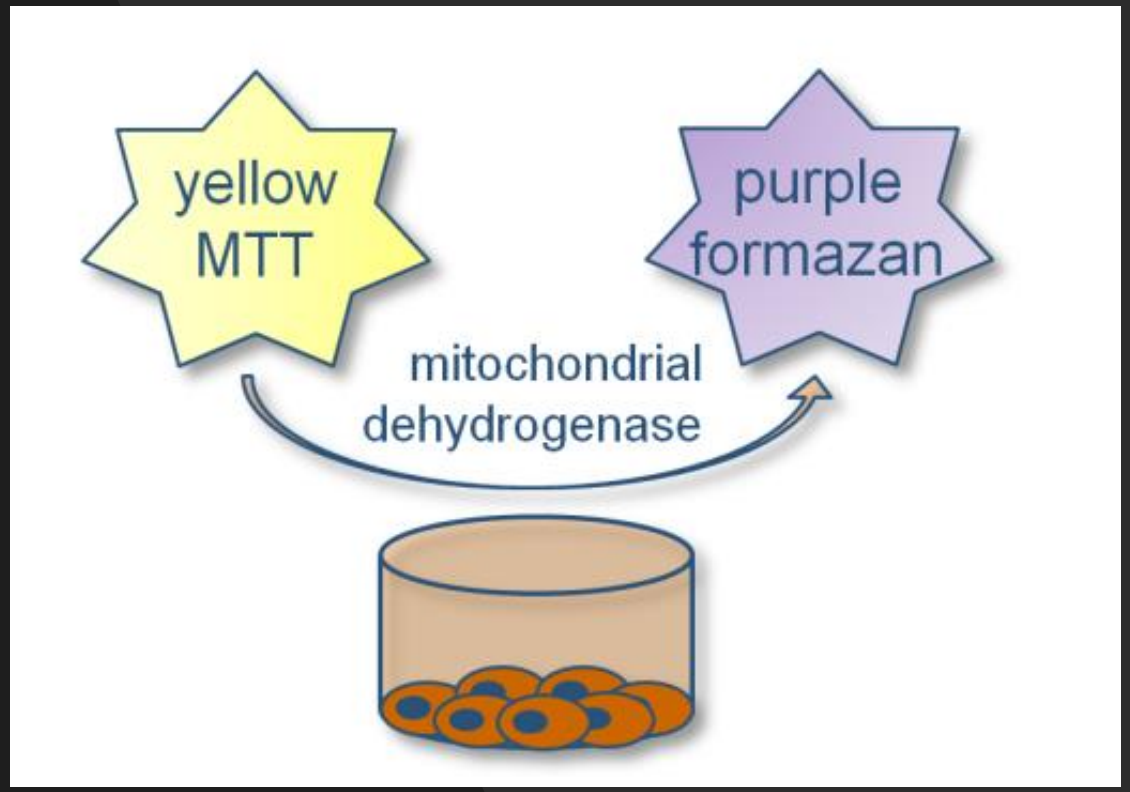

mode SE
HV 5.00 kV
spot 3.0
mag 2 519 x
det ETD
WD 14.1 mm
20 µm

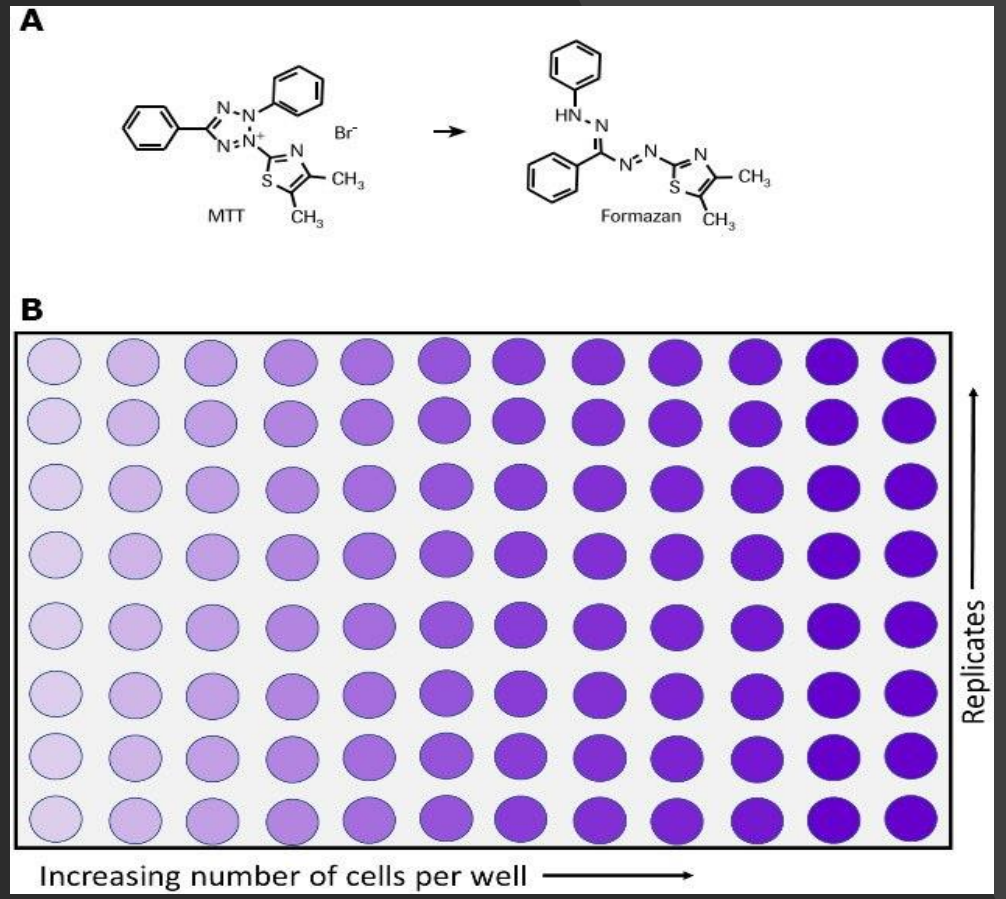


MTT Assay for Cell Viability

(using MG-63 Osteosarcoma cells)

- Cell mitochondria convert MTT into formazan crystals, causing a change in color.
- This allows for mitochondrial activity to be directly determined by an absorbance spectrum.





van Meerloo J., Kaspers G.J.L., Cloos J. (2011) Cell Sensitivity Assays: The MTT Assay. In: Cree I. (eds) Cancer Cell Culture. Methods in Molecular Biology (Methods and Protocols), vol 731. Humana Press. https://doi.org/10.1007/978-1-61779-080-5_20
https://www.sigmaaldrich.com/US/en/technical-documents/protocol/cell-culture-and-cell-culture-analysis/cell-counting-and-health-analysis/cell-proliferation-kit-i-mtt
https://www.eurofins.de/medizinprodukte-pruefungen/validierte-methoden/mtt-test/

Plated cells (control) →

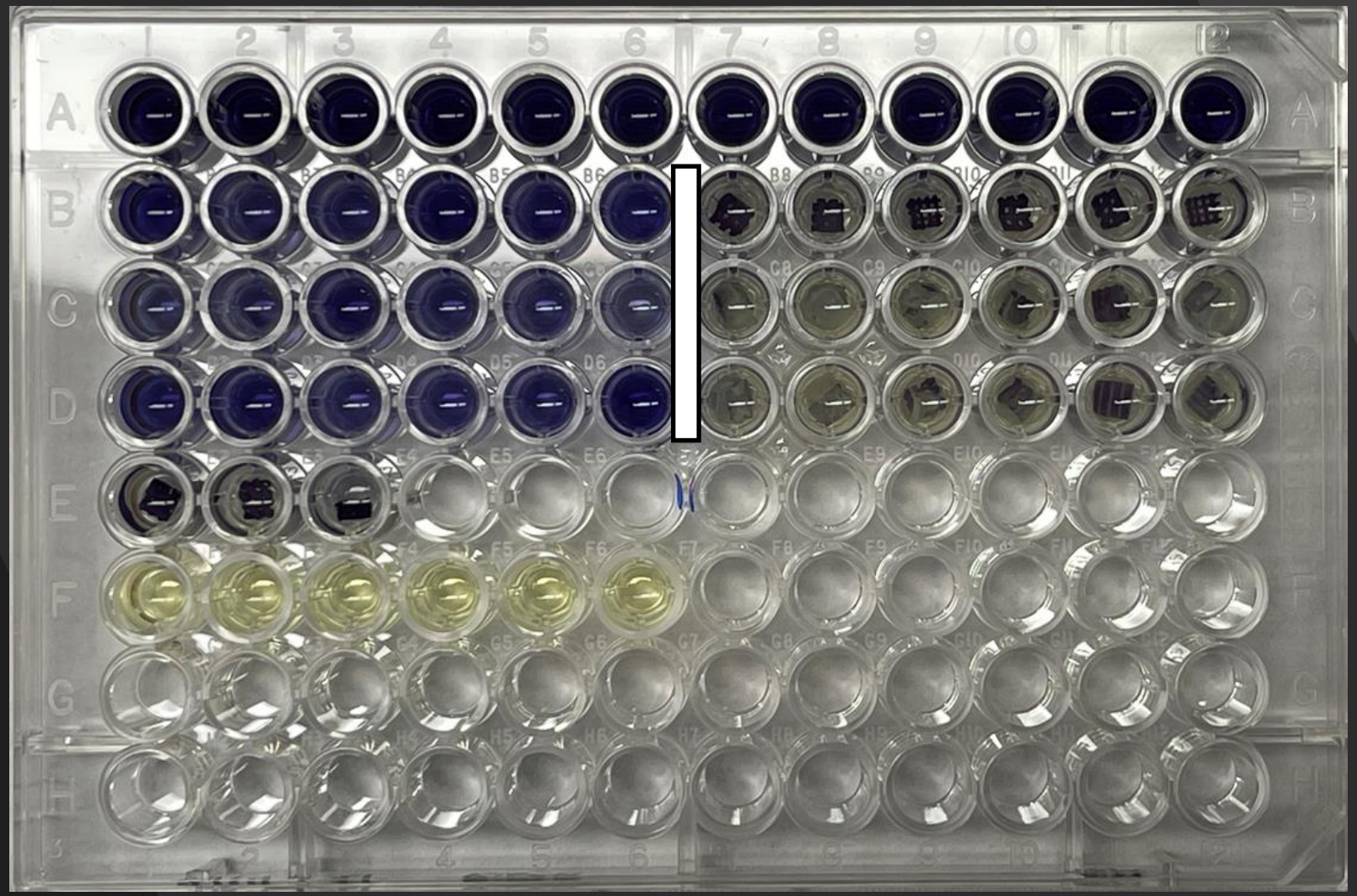
w/ GelMA →

N_2 treated w/ GelMA →

O_2 treated w/ GelMA →

Plain PCL (Control) →

Blank (No cells) →



← w/ GelMA-HA

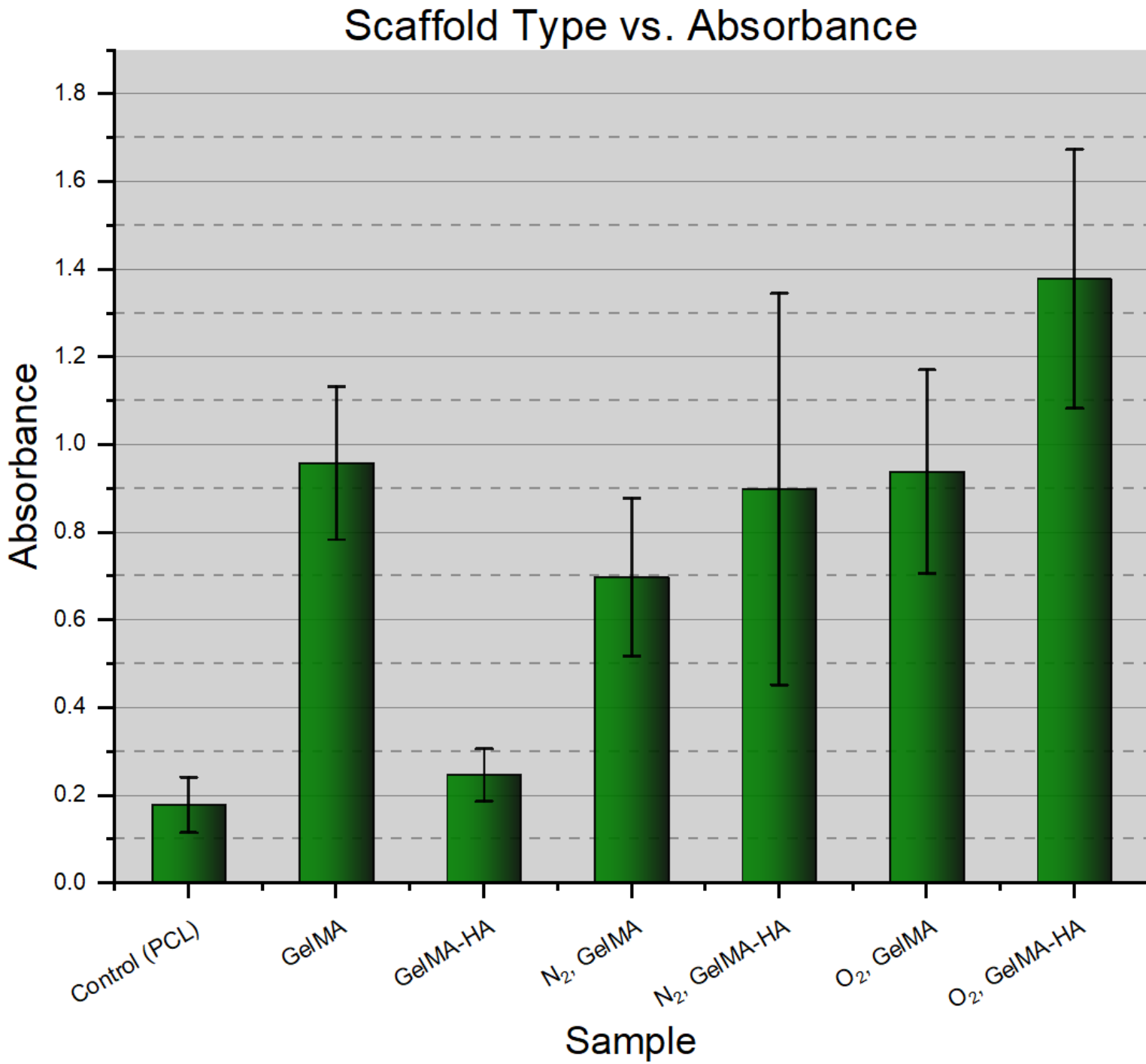
← N_2 treated w/ GelMA-HA

← O_2 treated w/ GelMA-HA

Higher absorbance

=

Greater cell viability



Summary

- What we did
  - Produced a unique type of scaffold for bone regeneration
  - Successfully integrated hydrogel with scaffold
  - Discovered unforeseen, adverse effects of LTP treatment on mechanical properties
  - Showed an unexpected increase in cell viability for GelMA over GelMA-HA
  - Successful, interesting questions, should be explored further

- What I learned
  - SolidWorks CAD software
  - 3d-printing
  - Analysis techniques
    - X-Ray Photoelectron Spectroscopy (XPS)
    - Water Contact Angle
    - Fourier-Transform Infrared (FTIR) Spectroscopy
    - Rheology
    - Scanning Electron Microscopy (SEM)
  - Hydrogel synthesis
  - Electrospinning
  - OriginLab plotting software

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